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United States
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Minutes

Agricultural Biotechnology Research Advisory Committee

Classification of
Unmodified Organisms
Working Group

June 22-23, 1989

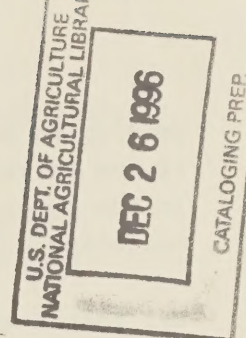


**United States
Department of
Agriculture**



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U.S. Department of Agriculture
Agricultural Biotechnology Research Advisory Committee
Working Group on Classification of Unmodified Organisms
Minutes of Meeting
June 22-23, 1989



The Agricultural Biotechnology Research Advisory Committee (ABRAC), Working Group on Classification of Unmodified Organisms (henceforth referred to as the Working Group) met on June 22, 1989 in Washington, D.C. Dr. Fred Gould chaired the meeting. The meeting was open to the public and had been announced in the Federal Register.

Members of the working group in attendance were: Dr. Fred Gould, Chair, Dr. Ronald Sederoff, Dr. A. Ann Sorensen, Dr. Rodney Bothast, Dr. Sue A. Tolin, and Dr. John D. Kemp (Attachment 1). Persons in attendance from the Office of Agricultural Biotechnology (OAB) were Alvin L. Young, Daniel D. Jones, Maryln K. Cordle, Barry E. Stone, and Lisa Zannoni.

Others in attendance were: Dr. Phillip O'Berry, Agricultural Research Service, Jo Anne Welsch, National Audubon Society, Susan Ely, ICI Seeds, John McClelland, USDA, Economic Research Service, Joseph McDade, Centers for Disease Control, Jane Rissler, National Wildlife Federation, Charles J. Eby, Monsanto, Monica Ter-Minassian, National Wildlife Federation, John R. Wood, USDA, Animal and Plant Health Inspection Service (APHIS) and Althaea Langston, APHIS.

I. CALL TO ORDER AND PRELIMINARIES

Dr. Gould called the meeting to order and asked the Working Group to review the agenda. The Working Group approved the agenda unanimously. Dr. Gould then asked Ms. Cordle to read the statement of charge to the Working Group.

Ms. Cordle indicated that a number of questions had been raised in outside comments on the May draft of the Guidelines for Research with Genetically Modified Organisms Outside a Contained Facility, (henceforth referred to as the Guidelines). In particular, questions were raised concerning the scientific rationale for assigning unmodified organisms to safety categories one through five in Table 1 of the Guidelines. The focus of the Working Group's charge, then, was to refine the scientific rationale for classifying unmodified organisms and assigning them to safety categories.

Dr. Gould asked Dr. Young if there was anything he would like to say to the Working Group. Dr. Young informed the Working Group that a decision had been made to

develop an Environmental Impact Statement (EIS) for the Guidelines. He added that, if the Working Group is successful in accomplishing its mission, OAB would publish in the Federal Register a "Notice of Intent" to prepare an EIS along with the draft Guidelines.

Dr. Kemp asked Dr. Young how long it would take to develop the EIS. Dr. Young stated that EIS development was a long process involving scoping sessions at multiple sites across the country and extensive documentation of the potential environmental effects of research under the Guidelines as well as various alternatives. He indicated that the whole process would take a year or more.

IV. REVIEW OF COMMENTS ON CLASSIFICATION OF UNMODIFIED ORGANISMS.

Ms. Cordle turned the group's attention to the comments received from the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the United States Department of Agriculture's Marketing and Inspection Services (MIS) and her summary of these comments. Ms. Cordle explained that she tried to extract the key points of each of the comments and organize them in line with the categories in the Guidelines so that one would not have to shift back and forth to see who spoke to which points.

Dr. Gould pointed out that one of the comments suggested that the problem of categorization cannot be solved by better examples adding that the information is insufficient for assigning an organism to a safety category. He then distributed comments from ABRAC members that were gathered from pre-meeting interaction among themselves as a starting point for discussion.

Dr. Kemp suggested a different approach for classifying organisms. Rather than simply assigning an organism per se to a safety category, he suggested using a series of attributes of the organism. The attributes could be assigned a value from one to five according to perceived risk and then an overall value for the organism could be determined. Organisms for which there is little information available would tend to be placed in a higher category until further information becomes available. Dr. Kemp made reference to a similar approach in a paper by Tiedje, et al., published by the Ecological Society of America (Attachment 2).

Dr. Sederoff suggested the placement of organisms in categories based on underlying principles. Dr. Kemp concurred saying that the researcher would have the responsibility to decide which attributes are most important for his particular

experiment. He added that the IBC would have the responsibility of placing the organism on a continuum of risk and documenting its decision.

Dr. Tolin advocated that the group first categorize the organisms which are currently being genetically modified since those are the ones for which more information is available.

Dr. Gould agreed and asked that the group come up with a list of attributes to classify organisms currently being researched. He then asked Dr. Kemp to present his ideas on the list of attributes he developed from the paper by Tiedje, et al. (Attachment 2).

Dr. Kemp presented his list of 10 attributes condensed from the Tiedje paper which included: 1) domestication; 2) need for subsequent control; 3) ease of subsequent control; 4) established or cultivated in release area; 5) inter-relationship with other organisms in the release environment; 6) pest/pathogen status (genetic exchange with pests/pathogens, potential to become a pest/pathogen, and potential to become a more serious pest/pathogen); 7) survival and persistence in the environment into which it is released (including long term spores); 8) genetic stability / intrinsic ability to mutate; 9) potential for genetic exchange; and 10) host range / geographic range / niche breadth.

After extensive discussion of the organism attributes listed by Dr. Kemp, and related information, the Working Group developed the following procedure as a generic tool for aiding the process of classifying unmodified organisms to levels of concern between 1 and 5.

ACTION I. Describe the accessible environment.

ACTION II. Describe the relevant aspects of the organism in the accessible environment.

ACTION III. Evaluate the relative importance of specific attributes. Carefully evaluate all of the attributes and explain why some are more or less important in the context of the planned test.

ACTION IV. Determine the safety condition of the unmodified organism. Provide a rationale for assigning the unmodified organism to safety conditions one through five as described below.

Under Action II, description of the relevant aspects of organism in the accessible environment, the Working Group developed the following attributes and descriptions.

1. Pest/pathogen status.

- a) Possible adverse effects of the organism on the accessible environment due to pest status or pathogenicity.
- b) Potential for exchange of genetic information between the organism and pest/pathogens in the accessible environment.
- c) Ecological characteristics of the organism that allow or inhibit it from becoming a pest or increasing in pest/pathogen status.
- d) Other.

2. Potential of the unmodified organism to establish itself in the accessible environment.

- a) Known mechanisms of survival or persistence in the natural environment.
- b) Known mechanisms of dissemination.
- c) Effects of population size of the organism on establishment.
- d) Competitive/aggressive behavior of the organism in the accessible environment.

3. Ecological relationships with other organisms.

- a) Role of the unmodified organism in the biological community and its importance.
- b) Ecological specificity/niche breadth. Extent to which the unmodified organism occupies a broad or narrow range or differing habitats.
- c) Extent of geographic range.

d) Habit (free-living, mutualistic, pathogenic, parasitic, symbiotic, etc.)

4. Potential for introduction of genetic change in natural or managed ecosystem.

5. Potential for control.

a) Prior research experience on control methods.

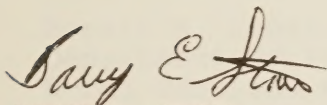
b) Monitoring methods.

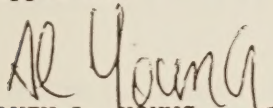
c) Handling of inadvertent release.

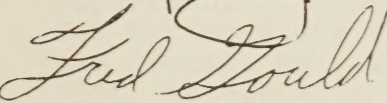
d) Other.

Several members of the Working Group agreed to prepare specific examples of organism classification as illustrative examples of how the above procedure might operate. These examples as well as a more detailed description of the classification procedure are included in Attachment 3.

Dr. Gould adjourned the meeting.


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Rapporteur


ALVIN L. YOUNG
Executive Secretary


FRED GOULD
Chairman

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THE RELEASE OF GENETICALLY ENGINEERED ORGANISMS: A PERSPECTIVE
FROM THE ECOLOGICAL SOCIETY OF AMERICA

Special Feature

THE PLANNED INTRODUCTION OF GENETICALLY
ENGINEERED ORGANISMS: ECOLOGICAL CONSIDERATIONS
AND RECOMMENDATIONS

JAMES M. TIEDJE, ROBERT K. COLWELL, YAFFA L. GROSSMAN,
ROBERT E. HODSON, RICHARD E. LENSKI, RICHARD N. MACK, AND PHILIP J. REGAL

Special Feature

The Release of Genetically Engineered Organisms: A Perspective From the Ecological Society of America

Recent advances in molecular and cellular biotechnology have made possible the production of transformed viruses, microorganisms, plants, and animals for use in industry, agriculture, health care, and other fields. Many of these new organisms will be designed for introduction and use in the environment. Biotechnology products have developed to the extent that a number of organisms are ready for field testing. For example, field tests have already taken place of transformed bacteria (*Pseudomonas syringae* and *P. fluorescens*) from which the gene for the ice-nucleation protein had been deleted, and a transgenic tobacco plant containing an inserted gene that confers resistance to the herbicide glyphosate. As noted by the Scientific Committee on Problems of the Environment and the Committee on Genetic Experimentation (SCOPE/COGENE, Bellagio, 22 September 1987), "the environmental introduction of any organism should be undertaken within a framework that maintains appropriate safeguards for the protection of the environment and human health while not discouraging innovation." SCOPE/COGENE, the National Academy of Sciences, and numerous decision makers have actively sought guidance from an ecological perspective on the issues involved in environmental introduction.

This Special Feature, "The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations," was prepared for the Public Affairs Committee of the Ecological Society of America. A workshop chaired by Dr. James Tiedje, Michigan State University, held 28–29 April 1988, in Washington, D.C., examined ecological aspects of the introduction of genetically engineered organisms into the environment. After initial drafting by the Workshop Committee, the report was extensively reviewed by the Public Affairs Committee and the Executive Committee and other ecologists knowledgeable in various areas of biotechnology. The final version, approved by the Public Affairs Committee and the Executive Committee, represents a service of the Ecological Society of America to practicing scientists and decision makers in the United States and elsewhere. The Society is grateful for the contributions of many members in this endeavor.

The report supports the use of advanced biotechnology for the development of environmentally sound products, and states that the phenotype of a transgenic organism, not the process used to produce it, is the appropriate focus of regulatory oversight. Ecological risk assessment of proposed introductions must consider the characteristics of the engineered trait, the parent organism, and the environment that will receive the introduced organism. A recommendation for the scaling of regulatory oversight by the attributes of organisms and environments is presented and represents the first development of a comprehensive overview of attributes that may permit a priori scaling of such oversight.

Members of the Ecological Society of America, colleagues in other disciplines, and decision makers at the federal, state, and local levels will find the Special Feature an important guidepost in this complex area. Interdisciplinary communication is particularly important as the field of biotechnology develops and expands. Careful design of transgenic organisms, along with proper planning and regulatory oversight, will ensure that these new organisms will pose little or no ecological risk.

H. A. MOONEY—*President*

P. G. RISSE—*Chair, Public Affairs Committee
Ecological Society of America*

Key words: biotechnology; field tests; fitness; genetic engineering; persistence; release of transgenic organisms; risk assessment; scientifically based regulation; species displacement.

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THE PLANNED INTRODUCTION OF GENETICALLY ENGINEERED ORGANISMS: ECOLOGICAL CONSIDERATIONS AND RECOMMENDATIONS¹

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Abstract. The ecological and evolutionary aspects of planned introductions of transgenic organisms into the environment are considered in this report. The authors support the timely development of environmentally sound products, such as improved agricultural varieties, fertilizers, pest control agents, and microorganisms for waste treatment, through the use of advanced biotechnology within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management. Economic, social, and ethical concerns also must be weighed along with strictly ecological and evolutionary considerations, but these other issues are beyond the scope of this report.

Ecological oversight of planned introductions should be directed at promoting effectiveness while guarding against potential problems. The diversity of organisms that will be modified, functions that will be engineered, and environments that will receive altered organisms makes ecological risk evaluation complex. While we cannot now recommend the complete exemption of specific organisms or traits from regulatory oversight, we support and will continue to assist in the development of methods for scaling the level of oversight needed for individual cases according to objective, scientific criteria, with a goal of minimizing unnecessary regulatory burdens. In this report, we provide a preliminary set of specific criteria for the scaling of regulatory oversight.

Genetically engineered organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them. Nonetheless, because many novel combinations of properties can be achieved only by molecular and cellular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques. Although the capability to produce precise genetic alterations increases confidence that unintended changes in the genome have not occurred, precise genetic characterization does not ensure that all ecologically important aspects of the phenotype can be predicted for the environments into which an organism will be introduced.

Many important scientific issues must be considered in evaluating the potential ecological consequences of the planned introduction of genetically engineered organisms into the environment. These include survival and reproduction of the introduced organism, interactions with other organisms in the environment, and effects of the introduced organism on ecosystem function. We encourage the use of small-scale field tests, when justified by previous laboratory and/or greenhouse studies, under conditions that minimize dispersal and under appropriate regulatory oversight. As the biotechnology industry develops, continuing regulatory oversight as well as long-term research and monitoring will be necessary for responsible risk management.

Many engineered organisms will probably be less fit than the parent organism, although some important exceptions may arise. Even if an engineered trait reduces an organism's fitness only slightly, many generations may pass before the introduced organism disappears completely due to decreased fitness. Such persistence is most probable when the turnover rate of populations is very slow.

Natural selection will act on genetically engineered organisms, as it does on all others. Selection after the release of the transgenic organism will tend to increase fitness, not

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decrease it, by reducing the costs associated with the novel traits. If increases in fitness do occur, they will probably increase population growth rate and biological competitiveness, or produce other ecological effects that should be considered in assessing risks.

Transfer of engineered genes from the modified organism to other organisms may occur through hybridization in higher organisms, or through conjugation, transduction, or transformation in microorganisms. If lateral transfer occurs, an engineered gene may persist in the natural environment even after the genetically engineered organism itself is no longer present. The available scientific evidence indicates that lateral transfer among microorganisms in nature is neither so rare that we can ignore its occurrence, nor so common that we can assume that barriers crossed by modern biotechnology are comparable to those constantly crossed in nature.

Native species, as well as species newly introduced from distant habitats, may become pests. An organism engineered to prosper in a new habitat type, geographic area, or season is effectively an introduced organism in that it will probably enter into new biotic and abiotic interactions. Therefore, regulatory and risk assessment structures that rely on the distinction between "native" and "non-native" must be used with caution.

Concern has frequently been expressed regarding the potential for genetically engineered organisms to displace resident species in the receiving community, particularly microbial species performing key functional roles such as nitrogen fixation or lignin decomposition. Because redundancy of function appears to be common in microbial communities, in many cases there would be little concern over microbial species displacement caused by an introduced transgenic organism.

Ecological effects and the geographic ranges of organisms transcend political boundaries; we therefore consider it essential to promote and achieve international coordination of risk assessment and regulation of biotechnology. Special consideration must be given to the protection of rare genetic resources, such as the wild ancestors of domesticated species, and threatened gene pools of other wild species. We urge local, state, national, and international cooperation in risk assessment and regulation of the ecological effects of the introduction of transgenic organisms.

Evaluating the benefits and risks of biotechnology products requires expertise in many scientific disciplines including molecular biology, genetics, cell biology, evolutionary biology, physiology, population and community ecology, and ecosystem science. For society to realize the full benefits of biotechnology, interdisciplinary research and graduate training programs are needed to expand the expertise of the scientific community at large.

INTRODUCTION

The ecological and evolutionary aspects of the planned environmental testing and large-scale use of genetically engineered organisms are considered in this report. New techniques of molecular and cellular biology, including recombinant DNA and cell fusion, have emerged as powerful research tools in biology. As the foundations of biotechnology, these techniques hold great promise for the development of new products derived from plants, animals and microorganisms to be employed in industry, agriculture, and health care.

Scientific issues involved in the introduction of genetically engineered organisms (also termed transgenic organisms) into the environment are addressed in this report. They include the potential effects of genetically engineered organisms on community and ecosystem processes, the effects of added genes on fitness, the potential for unintended transfer of genes to other species, the effects of scale and frequency of introductions, the applicability of several models for assessing

the potential ecological consequences, and a proposal for the scaling of regulatory oversight.

The writers and reviewers of this report, most of whom are members of the Ecological Society of America, represent the wide spectrum of professional ecological and evolutionary expertise encompassed by the 6500 members of the Society. **Although we support the timely development of environmentally sound products through the use of advanced biotechnology, we believe that these developments should occur within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management.**

The issues involved in the environmental introduction of transgenic organisms are complex and interdisciplinary. The development of effective biotechnology products and the assessment of their benefits and risks require expertise from many disciplines, in both natural and social sciences. This report is limited in scope to consideration of the ecological and evolutionary aspects of planned environmental introduc-

tions of genetically engineered organisms. Economic, social, and ethical concerns also must be weighed along with strictly ecological and evolutionary considerations.

Molecular and cellular biotechnology (including recombinant DNA, electroporation, projectile insertion, nuclear microinjection, and cell fusion) offers the potential for reaching many of the same goals pursued by traditional methods of plant and animal breeding and by mutation and selection of microorganisms, but in a more precise and efficient manner. For example, plant breeders have long sought to increase the disease resistance of crops through selection of resistant varieties and by hybridizing crops with wild relatives (Mayo 1987). Molecular techniques now permit the direct and precise introduction of genes from wild relatives, and cellular methods allow screening for the desired phenotype to proceed more efficiently. In addition to allowing the more rapid and precise accomplishment of such traditional objectives, however, molecular techniques now permit the integration of genetic information from very distantly related organisms, producing genotypes that no previous technology could ever have produced (e.g., Jaynes et al. 1987, Wong et al. 1988).

Novelty of a genotype, in itself, is not remarkable: every human embryo, unless an identical twin, has a genotype new to the planet. The novelty of biotechnology is its ability to exploit the universality of the genetic code to combine, in a single organism, major adaptive traits developed by organisms that have evolved along separate phylogenies. Organisms with novel combinations of traits are more likely to play novel ecological roles, on average, than are organisms produced by recombining genetic information existing within a single evolutionary lineage.

One prominent example of the new ability to combine traits from unrelated organisms is illustrated by the incorporation of a gene for the endotoxin of the bacterium *Bacillus thuringiensis* (or "*Bt*"), a registered microbial insecticide, into the genome of crop plants to protect them from insect damage (Goodman et al. 1987). For example, even though a tomato plant with an inserted *Bt* gene is most definitely still a tomato plant, its properties—as an organism, as a component of an agricultural ecosystem, and as a producer of human food—warrant investigation, because they may or may not parallel the properties of plants produced by traditional breeding techniques.

In addition to the ability to move genes among distantly related organisms, molecular techniques permit the incorporation of DNA sequences that code for completely novel proteins designed by the experimenter. For example, entirely synthetic proteins have been designed to supplement the overall production of

essential amino acids in potatoes. The appropriate DNA sequence is first designed and synthesized in the laboratory, then inserted into the potato DNA, along with sequences to regulate the expression of the new protein. This technique is already yielding transgenic potatoes of considerable promise for the alleviation of human malnutrition (Jaynes et al. 1986), but much work remains to be done with these transgenic varieties to assess their nutritional characteristics, pest and disease tolerance, role in agricultural ecosystems, and hazards of hybridization with wild relatives. Some of these assessments are routine for new varieties produced by traditional plant breeding, but others are genuinely new.

What ecology offers to the success of biotechnology

Professional ecologists are dedicated to the scientific study of interactions of organisms with one another and with the physical environment. Their work ranges from the study of the behavior and physiology of individual organisms in their environments, through the study of biological populations (single species) and interacting communities (many species), to the study of ecosystem function (biogeochemical transformations of energy and materials). The contributions that the field of ecology can offer biotechnology stem from the expertise derived from studying nature at these different levels of organization. A recent report published by the National Academy of Sciences details contributions made by the field of ecology to fisheries, wildlife, and forest management; pest control; and human health and welfare (National Research Council 1986a).

The National Academy of Sciences report, *Introduction of Recombinant DNA-Engineered Organisms Into the Environment: Key Issues* (1987), calls upon ecologists to provide guidance in evaluating planned introductions. Understanding ecological interactions is crucial to achieving the desired outcome of environmental introductions of transgenic organisms, including crop plants, pest control products, and beneficial microbial populations.

An example of past contributions of ecologists to environmental management can be drawn from early problems associated with pesticide use (Woodwell 1967, Woodwell et al. 1967). Ecologists responded to these problems by examining the transfer and concentration of pesticides in food chains and the evolution of pesticide resistance in target and nontarget organisms. Elucidating these processes resulted in the modern recognition of the value of biological control and the development of pest management programs based on the dynamics of pest populations, the introduction and preservation of beneficial organisms, and reductions in the amounts of the pesticides used (Huffaker 1980, National Research Council 1986b).

Ecological expertise will be important in future applications of biotechnology. The acquisition of effective gene libraries for use in biotechnology will be aided by recognizing and analyzing the products, functions, and evolutionary features of organisms in nature. For example, novel genetic defenses for crop plants may be obtained from the natural defenses of wild plants against insect pests. The microbial community also represents a largely untapped resource of useful genes. In addition, by investigating microbial competition and the necessary environmental conditions for population establishment, microbial ecologists will be able to assist in the successful establishment of populations of beneficial microorganisms. **The characterization and understanding of the ecological traits and requirements of transgenic organisms will often be critical to the development of successful, low-risk, genetically engineered organisms.**

Potential ecological effects

Most engineered organisms will probably pose minimal ecological risk. Many genetically engineered organisms will be modified domesticated species living under controlled agricultural conditions. Although domesticated animals sometimes establish feral populations, it would be difficult to convert most crop plants into organisms that can survive and reproduce without human support. However, in cases where an organism may persist without human intervention, or where there may be genetic exchange between a transformed organism and unaltered organisms, an assessment of environmental risk is required.

The following examples provide an overview of the types of undesirable outcomes to be avoided:

- 1) The creation of new pests. An example might be the creation of a salt-tolerant transgenic rice capable of escaping cultivated fields and invading estuaries.
- 2) Enhancement of the effects of existing pests through hybridization with related transgenic crop plants. One hypothetical class of examples would include the acquisition by weeds of engineered disease or herbicide resistance. Traits that enhance the competitiveness or physiological tolerances of weeds, such as salt, drought, or insect tolerance, will warrant consideration (Windle and Franz 1979).
- 3) Harm to nontarget species. For example, viruses with broadened host range could infect beneficial insects as well as the targeted pest.
- 4) Disruptive effects on biotic communities. The elimination of wild or desirable naturalized species through competition or interference is a possible outcome in some cases. For example, the introduction of the highly competitive nitrogen-fixing bacterium, *Bradyrhizobium* serogroup 123, into agricultural fields has made it difficult to introduce more effective rhizobia

(Ellis et al. 1984, Moawad et al. 1984). This negative economic outcome illustrates the importance of ecological information to sound economic analysis. Fish engineered to be larger or more cold tolerant also could have disruptive effects by displacing other fish species (Moyle 1986). The introduction of genes coding for an insecticidal toxin into forest or semi-domesticated tree crops (McGranahan et al. 1988) has the potential to alter plant community composition by diminishing seed predation, if the toxin is expressed in the seeds of the transgenic trees or in the seeds of wild relatives that have acquired the toxin genes by hybridization (Simmonds 1976).

5) Adverse effects on ecosystem processes. For example, the increased expression of microbial ligninase or constitutive denitrification could alter nutrient cycling adversely. Plants introduced for beneficial purposes may have adverse effects on ecosystem function, such as the disruptive effects on nitrogen fixation by the introduction of *Myrica* in Hawaii (Vitousek 1986). The availability of limiting nutrients may also be altered.

6) Incomplete degradation of hazardous chemicals leading to the production of even more toxic by-products. For example, the microbial degradation of trichloroethylene (TCE) and tetrachloroethylene (PCE) produces the more toxic vinyl chloride (Vogel and McCarty 1985).

7) Squandering of valuable biological resources. For example, the genes for toxins produced by strains of the bacterium *Bacillus thuringiensis* (*Bt*) have now been inserted into several crop plants and trees, conferring resistance against some insect herbivores. However, the genetically engineered crops as well as the unaltered bacteria could be rendered ineffective by creating conditions that accelerate the evolution of pest resistance (Gould 1988a, b, Colwell, *in press*).

Transgenic organisms can be designed to minimize the chance of environmental perturbations. The choice of the trait and parent organism used, the form of the genetic alteration, and the control of its expression all affect the likelihood that the genetically engineered organism will have undesirable effects. In addition, the conditions of the organism's introduction can be planned to minimize potential problems. Thus, we believe that with careful design of transgenic organisms and proper planning and regulatory oversight of environmental releases, the introduction of many transgenic organisms can be carried out with minimal ecological risk.

SCIENTIFIC ISSUES

The complexity that arises in evaluating the efficacy and possible risks of environmental uses of biotechnology stems from the diversity of organisms to be

modified, the desired functions to be introduced, and the physical and biotic components of target environments. The organisms that may be engineered span the entire range of organisms from viruses, bacteria, and simple eukaryotes to multicellular plants and animals, including domesticated and wild species. The expertise necessary for evaluating the benefits and risks of biotechnology involves many scientific disciplines, including molecular biology, genetics, cell biology, evolutionary biology, physiology, population and community ecology, and ecosystem science (Hodgson and Sugden 1988).

The pending release of genetically altered organisms into the environment raises an array of potentially important scientific issues. The survival and reproduction of transgenic organisms, their interactions with other organisms, their potential for spread beyond the point of their introduction, and their effects on the physical environment will all individually and collectively influence whether the released organisms pose any risk to the environment. Knowledge of the genetics, physiology, and ecology of both the parent organism and the gene donor, and the characteristics of the environment that will receive the modified organism will be necessary for an evaluation of the potential effects of a transgenic form (National Institutes of Health 1985, National Academy of Sciences 1987).

Importance of phenotype

Some transgenic organisms will differ little from the parent organism, perhaps by only one gene. Others will differ much more. The important consideration is the character of the phenotypic changes expressed after manipulation of the organism's genotype, not simply how many genes have been added or deleted. Changing a market-oriented attribute of an agricultural plant, such as the amino acid content (for example, in corn), may have few important ecological consequences. On the other hand, phenotypic changes that result in increases in physiological tolerance, substrate utilization, or range extensions may have far-reaching effects on the phenotype's abundance, range, and interactions with other organisms and with the physical environment.

We contend that transgenic organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them. Both the intended product-oriented phenotypic changes and other, possibly unintended, changes in the phenotype need to be considered. The new phenotype of the transgenic organism, together with the characteristics of the environment into which it will be introduced, should be the basis for evaluating the potential ecological effects of a proposed introduction. Nonetheless, because many novel combinations of properties can be achieved only

by molecular and cellular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques.

Characterization of the genetic change

Traditional methods of genetic alteration often produce multiple, unknown effects on the genotype and unexpected effects on the phenotype. In contrast, the precision with which genetic alterations can be made with molecular techniques is often cited as an argument for the safety of genetically engineered organisms (Brill 1985, Davis 1987). Pleiotropic effects (secondary phenotypic effects of a single genetic alteration), however, may easily be overlooked in focusing on intended primary effects, and some effects may be expressed only in particular environments (e.g., Stotzky and Babich 1985), even if the genotype is fully characterized.

Although the capability to produce precise genetic alterations increases confidence that unintended changes in the genome have not occurred, precise genetic characterization does not ensure that all ecologically important aspects of the phenotype can be predicted for the environments into which an organism will be released.

Fitness of genetically engineered organisms

The potential for a transgenic organism to survive and reproduce in the environment for which it is intended must be examined when considering the possible long-term consequences of an introduction. It has often been assumed that the addition of extra genes reduces the competitiveness of an organism due to the added cost of synthesizing additional nucleic acids and proteins (e.g., DaSilva and Bailey 1986, Davis 1987, Lenski and Nguyen 1988). Normal physiological processes may also be disrupted as a result of the expression of novel traits. For example, Bassford et al. (1979) observed that the growth of bacterial cells synthesizing a *malE-lacZ* hybrid protein was severely impaired, apparently because the hybrid protein became embedded in the cytoplasmic membrane and thus blocked sites needed for transport of essential components of the outer membrane. Similarly, Moyed et al. (1983) found that high-level expression of a tetracycline resistance gene could severely inhibit bacterial growth. They demonstrated that the level of phenotypic resistance to tetracycline actually decreases at very high levels of expression of the resistance gene. This example illustrates the potential complexity of the relationship between genotype and phenotypic expression.

In contrast, there are also important exceptions to the general observation that additional genes decrease competitiveness. Devanas and Stotzky (1986) found that nonessential eukaryotic DNA inserted into plasmid DNA had little effect on bacterial survival in lab-

oratory soil or saline environments. In the first field test of an engineered soil bacterium (*Pseudomonas fluorescens* with inserted *lacZY* gene), the population sizes of the introduced parent and transgenic organisms were identical for 30 wk (Kline et al. 1988). Furthermore, competitiveness in bacteria has even been enhanced with the possession of foreign DNA (Hartl et al. 1983, Edlin et al. 1984, Bouma and Lenski 1988, Marshall et al. 1988). For example, Hartl and colleagues (1983) found that the transposon Tn5 and its associated IS50 insertion sequences actually increase the growth rate of certain bacterial strains. The precise genetic and physiological bases of these exceptions are not known, but they raise the possibility that similar fitness-enhancing functions may inadvertently be transmitted along with an intended genetic alteration during molecular engineering.

Even in cases where fitness is reduced, the transgenic organism may persist in the environment. For example, if there is a 1% reduction in growth rate per generation of the transgenic organism relative to the wild type, hundreds or even thousands of generations may be required for the population of the introduced organism to be completely displaced. This phenomenon may be especially problematic when the turnover rate of populations is very slow, as is likely for many higher organisms and microbial species growing at very low resource concentrations.

In summary, many engineered organisms will probably be less fit than the parent organism, although some important exceptions may arise. Even if an engineered trait reduces an organism's fitness only slightly, many generations may pass before the introduced organism disappears completely due to decreased fitness.

Variation and selection

If novel genes can enhance competitiveness, why have organisms not evolved these functions already? Current ecological and evolutionary thought does not embrace the notion that organisms are perfectly adapted to their environment (Gould and Lewontin 1979, Harper 1982). There are important constraints on evolutionary processes that limit the ability of organisms to become perfectly adapted. In particular, adaptation requires the existence of appropriate genetic variants on which selection can act. Much of the power of modern molecular biology lies in its ability to circumvent this constraint and produce radically new genetic variants.

Natural selection will act on transgenic organisms as it does on all others. **Selection after the release of the transgenic organism will tend to increase fitness, not decrease it, by reducing the costs associated with the novel traits.** The reduction in costs could result from a mutation that reduces the level of expression of a

costly gene product, or one that compensates for some critical physiological process disrupted by the engineered gene product. Such changes in the burdens associated with novel phenotypes have been documented in both microbes and higher organisms (Lenski and Nguyen 1988). Mutants of *E. coli* that are resistant to the virus T4 are less competitive than the virus-sensitive parental strain when the two strains are allowed to compete in the absence of the virus (Lenski and Levin 1985). However, this cost of resistance has been overcome, at least in part, by a subsequent adaptation enhancing competitiveness without reducing resistance (Lenski 1988). Similarly, McKenzie et al. (1982) have documented that a reduction in competitiveness in the sheep blowfly (*Lucilia cuprina*) associated with resistance to the insecticide diazinon, was eliminated by subsequent adaptation of the blowfly. If increases in fitness do occur in transgenic organisms introduced into the environment, they will probably increase population growth rate and biological competitiveness, or produce other ecological effects that should be considered in assessing risks.

Organisms bearing phenotypes new to a biological community may also act as agents of natural selection. Gould (1988a, b) modeled the evolution of resistance in insects to *Bacillus thuringiensis* (*Bt*) toxin following the introduction of agricultural cultivars carrying the gene for its production and found that the greater exposure to *Bt* toxin presents herbivorous insects with a powerful new selective pressure that may result in the acquisition of *Bt* resistance. Such resistance could reduce the efficacy of engineering the *Bt* toxin into agricultural cultivars and could also change the role that naturally occurring *Bt* toxin plays in the ecosystem.

Gene stability and transfer

Assessing the risks associated with the introduction of transgenic organisms is made more complex by the capacity of many modified organisms to exchange genes with unaltered organisms. For example, crop plants vary enormously in their potential for hybridization with wild relatives (Harlan 1965). At one extreme is the crop turmeric (a component of curries) which is maintained in cultivation entirely through vegetative propagation; at the other extreme is alfalfa, which is an obligate outbreeder (Harlan 1975). Other species such as peas, lentils, and wheat are considered inbreeders but are capable of some outcrossing. Despite the intensive search by plant breeders for the wild relatives of crops, knowledge of the distribution and the ecology of these wild species is surprisingly incomplete. We do know, however, that few major crops originated in temperate North America (Hodge and Erlanson 1956). Many crop species, exemplified by rice, tomato, and rubber, arose in tropical or subtropical areas where they

often are cultivated in fields adjacent to their wild relatives (Harlan 1975). Care should be taken to prevent crops from passing inserted genes to their wild relatives via hybridization. Such transfer becomes important because genes that confer a new ability, such as insect or disease resistance, or salt or drought tolerance, could also change the physiological tolerances or geographic distributions of wild plants, causing them to become economically important weeds or altering their roles in natural communities (Hauptli et al. 1985, Center for Science Information 1987, Ellstrand 1988).

Transgenic bacteria may exchange genetic material with naturally occurring strains by conjugation, transduction, or transformation (Lenski 1987, Miller 1988). If lateral transfer from the modified organism to other organisms occurs, an engineered gene may persist in the natural environment even after the genetically engineered organism itself is no longer present. This is one category of extenuating circumstances that will need to be considered in risk assessment.

Current knowledge of gene exchange by microbes comes largely from laboratory studies with a few vectors heavily used in molecular genetics. Little information exists on the prevalence of these vectors outside the laboratory. We know, however, that microbial taxa are variable in the extent to which they exchange chromosomal and extra-chromosomal genes (Lenski 1987), and that biological, physical, and chemical properties of the environment can all influence the rate of gene exchange (Stotzky and Krasovsky 1981). **The available scientific evidence indicates that lateral transfer among microorganisms in nature is neither so rare that we can ignore its occurrence, nor so common that we can assume that barriers crossed by modern biotechnology are comparable to those constantly crossed in nature.** Techniques are being developed to prevent or reduce the potential for gene transfer (Bej et al. 1988, Office of Technology Assessment 1988). By engineering a stable construct, verifying its stability in the presence of known vectors, controlling the densities of introduced organisms, and characterizing potential environments that will receive the transgenic organisms, field experiments with low risk for gene exchange are possible.

Effects of the scale and frequency of introductions on establishment

In some cases, the establishment of persistent populations of genetically engineered organisms will be the goal of an environmental introduction. For example, transgenic organisms intended for the biological control of particular pests may be designed to persist at low population levels in the absence of a pest outbreak. In other cases, engineered organisms will be designed to carry out a particular mission, then die out or recede to minimal population levels. Examples include the

breakdown of toxic waste by microbes (e.g., Omenn 1988), or the production of commercial products by annual plants (see Center for Science Information 1987). Whether establishment is intended or not, the ability of a genetically engineered organism to become established in natural or managed ecosystems becomes a critical issue.

Case histories of both disease epidemics and invasions of higher organisms suggest that the scale of the introduction can determine whether the introduction yields a self-sustaining population (e.g., Crowell 1973, Schoener and Spiller 1987). The minimum effective inoculum threshold varies widely among organisms both with regard to the density of the inoculum and the geographic range over which it is introduced (Dunigan et al. 1984, Simberloff 1986). Threshold numbers vary for different reasons. In some cases, rapid predation or another source of high mortality can be overcome only by a large founder population. In other situations, a large founder population will provide sufficient genetic variation from which genotypes can be selected that can tolerate the new environment (Salisbury 1961, Baker 1986).

The frequency of introductions also affects the ability of an organism to establish a population, because frequent releases increase the likelihood that a threshold number of individuals will find sites favorable for establishment and reproduction. Moreover, the season and environmental conditions at the time of introduction could influence whether the population will become established (Ridley 1930, Crawley 1988). The method of introduction also influences establishment: microorganisms injected into the xylem of plants, released as aerosols, or mixed with soils will experience very different initial conditions, resulting in different probabilities of establishment.

Under most circumstances, small-scale field tests will involve the introduction of a limited number of organisms into a limited environment, on one or a few occasions. These conditions will reduce the probability that a transgenic population will become established from a small-scale field test. **Thus we encourage the use of small-scale field tests, when justified by previous laboratory and/or greenhouse studies, under conditions that minimize dispersal and under appropriate regulatory oversight.** Field experimentation and data from population monitoring are also crucial in evaluating the potential for larger scale environmental effects.

Fate of introduced transgenic organisms: potential for delayed effects

If a transgenic organism can be eliminated following an experimental introduction, the risk associated with its field testing will usually be insignificant. Elimination may be practical for larger plants and animals. Insects,

microorganisms, and viruses, however, may be difficult to exterminate following their introduction. Difficulty or uncertainty about the extermination of an introduced organism should result in closer scrutiny of the proposed introduction.

The ability of organisms to persist in nature is often surprising, and we expect that cases of persistence of transgenic organisms will emerge. In a number of documented cases, introduced microorganisms were thought to have disappeared because they could not be detected by culture methods, but much later the progeny of these organisms or their gene sequences appeared under the appropriate conditions for growth (Devanas et al. 1986, Chatterjee 1988). In addition, there are a number of disease-causing organisms that are no longer detectable, yet the disease occurs (R. R. Colwell et al. 1985). Even among higher organisms, a small number of individuals, below the limits of detection, may persist for a period of time, then suddenly increase when appropriate environmental conditions occur (Mack 1985, Moody and Mack 1988). **Rather than focus upon whether an introduced transgenic organism is likely to disappear completely, emphasis should be placed on whether its population is likely to remain viable or increase in size under appropriate environmental conditions.**

Furthermore, many types of ecological effects may be indirect, taking some time to appear. Examples include bioaccumulation, species replacement, and perturbations of geochemical cycles that have been observed only after many years of chronic pollution or, in some cases, years after a single introduction of a new species into the environment. Unlike the effects of releasing chemicals into the environment, the direct effects of self-replicating introduced organisms may not necessarily decrease with time or with distance from the point of introduction. **The absence of an immediate negative effect does not ensure that no effect will ever occur.**

Community and ecosystem level effects

Establishment of persistent populations depends upon interactions between the engineered organism and other species in the biological community, and upon the integration of the organism into the receiving ecosystem. In turn, successful establishment will sometimes have significant effects on species interactions and ecosystem function (Williamson 1988). Although the focus of past discussions has often been on the capability of engineered organisms to compete successfully with naturally occurring organisms and on their potential for pathogenicity (e.g., Davis 1987), other classes of species interaction are also important. Mutualistic interactions, in particular, underlie many critical ecosystem functions, including nitrogen fixation,

inorganic nutrient uptake through mycorrhizae, pollination, cellulose decomposition, and digestive processes in both invertebrate and vertebrate animals (Futuyma and Slatkin 1983). **Engineered organisms that alter mutualistic associations, either by design or unintentionally, require careful evaluation.**

Initial information on the potential for establishment and possible effects of an engineered organism should be obtained from laboratory microcosm and mesocosm studies, or from tests in a contained greenhouse. Assuming these preliminary studies reveal no unacceptable risks, further information should be obtained from carefully planned small-scale field trials that include evaluation of both intended and unintended effects on other species in the ecosystem.

The widespread establishment or use of transgenic organisms might result in effects on ecosystem function. For example, if an improved *Bradyrhizobium* strain were to dramatically increase nitrogen fixation and enhance legume productivity, it is likely that soil nitrogen would be enriched because nitrogen mineralization would probably continue at its usual rate. Excessive soil nitrogen might then lead to establishment of new weeds, increased leaching of nitrate, and increased flux of nitrogen oxides into the atmosphere.

In microbial communities, we often understand the functional roles that microorganisms play better than we know the species composition of the community. Concern has frequently been expressed regarding the potential for introductions of transgenic organisms to displace resident species in the receiving community, particularly microbial species performing key functional roles such as nitrogen fixation, lignin decomposition, or pesticide degradation (Levin and Harwell 1986). Natural communities have considerable "redundancy" in that many species perform similarly in key roles. Such "functionally equivalent" species, however, undoubtedly differ ecologically in other respects. Differences in functional roles will be important in some cases, especially among higher organisms, but may be of little importance in others. For example, the value of a species at risk for displacement may be economic or aesthetic, in addition to any role it may play in community and ecosystem function. **Because redundancy of function appears to be common in microbial communities, in many cases there would be little concern over microbial species displacement caused by an introduced transgenic organism.**

The merits and limits of existing models

Agricultural breeding.—Numerous models have been used in evaluating the potential effects of the introduction of transgenic species into the environment (Goldburg 1988, Regal 1988, Colwell, *in press*). Most of these have some merit, but all have limits in their

applicability. One general model is based on the long-term experience derived from traditional breeding (e.g., Ellingboe 1985, National Academy of Sciences 1987). This vast and often anecdotal record provides useful information on the effects of moving domesticated species from one location to another, and on moving genes via hybridization to create new strains. This record provides useful information for the evaluation of genetic alterations similar to those that might have been produced by traditional means, and such alterations are likely to pose few ecological problems. The agricultural model, however, reflects techniques that lack the wide-ranging ability of molecular biology to transfer traits among very different species. Consequently, an overall record of little or no hazard stemming from the release of the products of traditional agricultural breeding does not legitimately warrant exemption from oversight for future introductions of transgenic organisms that these traditional techniques could not have produced.

Introduced species model.—The introduced species model provides another possible analog for the potential ecological effects of the environmental release of genetically engineered organisms (Sharples 1983, 1987, R. K. Colwell et al. 1985, Regal 1986). Most terrestrial and freshwater biological communities include self-propagating species from two general sources. Some are native species that were present before human intervention. Others are naturalized, that is, they were introduced either deliberately or accidentally by humans and have become integrated into the biological community. *Bradyrhizobium japonicum* (a symbiotic nitrogen-fixing bacterium of soybean), certain crops that have escaped from cultivation including Jerusalem artichoke and blackberries, many agricultural weeds, and a few animals including pheasants and brown trout, fit this category. Occasionally, these introduced species have become pests.

A functional analogy may be drawn between the reasons for the naturalization of non-native organisms and the possibility that introduced transgenic organisms might become naturalized. For example, a plant may persist in a new range because in the course of immigration it has escaped its native herbivores and has not attracted new herbivores. A transgenic plant may experience the same outcome with insertion of genes that control the production of proteinase inhibitors (Thornburg et al. 1987). Similar functional analogies could be drawn between the escape from fungal parasites or the extension of growing season, and the impending or contemplated insertion of genes imparting fungal resistance or frost tolerance. In drawing these functional analogies, we are not maintaining that all transgenic plants will become naturalized; we contend instead that our ability to assess this risk (of whatever size) will be

enhanced by realizing that under some circumstances imparting a new trait to a plant may confer the same advantages that some plants have experienced through their dispersal to areas far outside their usual ranges (Simberloff 1985, Williamson 1988). This analogy can also be applied to other groups of higher organisms.

An organism engineered to prosper in a new habitat, geographic area, or season is effectively an introduced organism in that it probably will enter into new biotic and abiotic interactions. Both native species and species introduced from distant habitats may become pests (Pimentel 1986). Regulatory and risk assessment structures that rely on the distinction between "native" and "non-native" must therefore be used with caution. **Historical experience with the introduction of non-native species may be relevant for modified native or naturalized organisms, as well as for the introduction of genuine non-natives.**

Laboratory experience.—A commonly cited argument for the safety of genetically engineered organisms stems from the laboratory experience gained since the mid-1970s with recombinant-DNA microorganisms. Although some of these organisms have probably escaped containment, negative effects have not been detected (Davis 1987). These escapes have probably ended in local extinction because the escapees arrived in incompatible habitats in numbers below the threshold densities for establishment, and because these organisms were often intentionally designed to have lower fitness than their genetically unaltered counterparts.

Although obtaining laboratory data is an important first step in evaluating the ecological traits of genetically engineered organisms, these data alone cannot accurately predict the fate of an introduced organism released in nature. The complexity and dynamic character of the physical, chemical, and biological environment has not been duplicated in the laboratory. Laboratory soil microcosms can provide reasonably natural habitats to study the ecological properties of a soil microorganism. Extensive experience in plant pathogen research, however, has shown that the laboratory is not a reliable environment in which to study the ecology of a leaf pathogen. Thus the usefulness of data from laboratory studies for predicting environmental fate will vary widely.

REGULATORY POLICY

Previous sections discussed scientific principles derived from the field of ecology that should be used in planning for the development, testing, and risk assessment of the introduction of genetically engineered organisms into the environment. Ecological oversight of such introductions should be directed at promoting effectiveness while guarding against potential problems. The diversity of organisms that will be modified,

functions that will be engineered, and environments that will receive modified organisms makes ecological risk evaluation complex. Clearly, however, different organisms, traits, and environments present different probabilities of adverse effects. This complexity has made it difficult to establish categories for different degrees of regulatory oversight. Ecological knowledge, however, does allow us to endorse certain principles that should be useful in developing regulatory policy and in recognizing the degree of risk associated with different attributes of engineered traits, organisms, and environments.

Scaling of regulatory oversight—a recommendation

Regulatory agencies are currently working to develop risk assessment guidelines and policies within the framework of a diverse set of legal mandates and historical precedents (National Institutes of Health 1985, 1986, Office of Science and Technology Policy 1986, McGarity 1987, Ager 1988, Kingsbury 1988, Office of Technology Assessment 1988). While we cannot now recommend the complete exemption of specific organisms or traits from regulatory oversight, we support and will continue to assist in the development of methods for scaling the level of oversight appropriate to individual cases according to objective, scientific criteria. The goal is to ensure safety while minimizing unnecessary or counterproductive regulatory burdens. In this section we attempt to outline a set of genetic, phenotypic, and environmental criteria that might provide a scientific basis for uniform policy. Table 1 presents a preliminary attempt at developing a comprehensive overview of attributes that may permit a priori scaling of regulatory oversight for the testing and use of genetically engineered organisms in the environment. (The design of this table was inspired by a similar table prepared by the Recombinant DNA Monitoring Committee [Australia] 1987.)

As we have stressed, ecological risk assessment and regulatory oversight of genetically engineered organisms, for either field testing or commercial use, require consideration of many factors. Some may be quantified, such as numbers of organisms to be released, size of test plots, or susceptibility of organisms to biocides should mitigation be necessary. Many factors are, however, necessarily qualitative or only roughly quantifiable, such as the level of domestication of the parent species, or the infectivity or virulence of the parent organism. Nonetheless, we consider it essential to develop some practical means of scaling the level of regulatory oversight according to the probability of adverse effects. Such scaling is equally essential as a means of focusing risk assessment on only those features of organisms and environments that require it.

Three features of Table 1 must be stressed. First, the

scaling of each attribute is only qualitative or semi-quantitative (ordinal). Second, the scales for any two attributes (any two lines in the table) are not necessarily commensurate, either in terms of the probability of adverse consequences or the severity of such an outcome, if realized. Third, the table is multidimensional. Overall estimates of risk for a particular genetically engineered organism in a particular environmental context require, in effect, a simultaneous mapping of the attributes of the case on all the individual scales of the table. The estimate of risk based on one scale may be greatly modified by the scores on other scales. For example, if the parent organism is a pathogen ("habit" scale in Part B), then a broadened host range ("host range" scale in Part C) will yield a higher estimate of risk than would a non-pathogenic organism with a comparably broadened host range.

We must warn against combining scores on the different scales of the table. Not only are they incommensurate, but scores on different scales are also neither strictly multiplicative (as fully independent probabilities would be), nor strictly additive. Experience with real cases will ultimately provide information on actual levels of risk for many regions of this multidimensional space.

In the meantime, we urge that any case that falls at the right-hand end of *one or more* scales in Table 1 should receive appropriate regulatory scrutiny in regard to the attribute(s) in question. Ecological safety, as well as public confidence in a fledgling industry, will be fostered by this approach. Clearly many in the biotechnology industry see the issue in much the same way that we do. In the words of Robert Goodman and his colleagues (Goodman et al. 1987) at Calgene, Inc.:

The unusual power of the technology, uncertainty over the behavior to be expected from organisms modified in novel ways, and the past 40 years of experience with chemicals in the environment make it reasonable and indeed desirable that genetically modified organisms be introduced cautiously.

Comments on current regulatory policy

The current regulatory framework uses biological characteristics and existing institutions (e.g., federal agencies, universities, industrial laboratories) to structure the oversight process (Office of Science and Technology Policy 1986). Certain aspects of the regulatory framework warrant comment from an ecological perspective.

Case by case review.—The current regulatory practice for review of proposed environmental introductions uses a case by case analysis of the organism, environment, and experimental protocol. **Case by case review is currently the most scientifically sound regu-**

TABLE 1. Attributes of organisms and environments for possible consideration in risk evaluation.*

A. Attributes of genetic alteration

	Level of possible scientific consideration		
	Less		More
Characterization	Fully characterized		Poorly characterized or unknown
Genetic stability of alteration	High (e.g., chromosomal)		Low (e.g., extra-chromosomal)
Nature of alteration	Gene deletions (unless host range altered)	Single gene added	Multiple genes added
Function	None (no expression or regulation)	Regulation of existing gene product	Synthesis of gene product new to parent organism
Source of insertion	Same species	Closely related species	Unrelated species
Vector	None	Non-self-transmissible	Self-transmissible
Source of vector	Same species; non-pathogen	Closely related species; non-pathogen	Unrelated species or pathogen
Vector DNA/RNA in altered genome	Absent	Present, but non-functional	Functional

B. Attributes of parent (wild type) organism

	Level of possible scientific consideration		
	Less		More
Level of domestication	Unable to reproduce without human aid	Semi-domesticated; wild or feral populations known	Self-propagating, wild
Ease of subsequent control	Control agents known		No known control agents
Origin		Indigenous	Exotic
Habit	Free-living		Pathogenic, parasitic, or symbiotic
Pest status	Relatives not pests	Relatives pests	Pest itself
Survival under adverse conditions	Short term		Long term (e.g., spores, cysts, seeds, dormancy)

TABLE 1. Continued.

Geographic range, range of habitats	Narrow	Broad or unknown
Prevalence of gene exchange in natural populations	None	Frequent

C. Phenotypic attributes of engineered organism in comparison with parent organism

	Level of possible scientific consideration		
	Less		More
Fitness	Reduced irreversibly	Reduced reversibly	Increased
Infectivity, virulence, pathogenicity, or toxicity	Reduced irreversibly	Reduced reversibly	Increased
Host range	Unchanged		Shifted or broadened
Substrate, resource	Unchanged	Altered	Expanded
Environmental limits to growth or reproduction (habitat, microhabitat)	Narrowed but not shifted		Broadened or shifted
Resistance to disease, parasitism, herbivory, or predation	Decreased	Unchanged	Increased
Susceptibility to control by antibiotics or biocides, by absence of substrate, or by mechanical means	Increased	Unchanged	Decreased
Expression of trait	Independent of environmental context		Dependent on environmental context
Similarity to phenotypes previously used safely	Identical	Similar	Dissimilar

D. Attributes of the environment

	Level of possible scientific consideration	
	Less	More
Selection pressure for the engineered trait	Absent	Present

TABLE 1. Continued.

Wild, weedy, or feral relatives within dispersal capability of organism or its genes	Absent	Present	
Vectors or agents of dissemination or dispersal (mites, insects, rodents, birds, humans, machines, wind, water, etc.)	Absent or controllable	Present, uncontrollable	
Direct involvement in basic ecosystem processes (e.g., nutrient cycling)	Not involved	Marginally involved	Key species
Alternative hosts (partners), if organism is involved in symbiosis (mutualism)	Absent	Present	
Range of environments for testing or use; potential geographical range	Very restricted	Broad, widespread	
Simulation of test conditions	Not difficult to simulate realistically	Very difficult to simulate realistically	
Public access to test site	Tightly controlled	Limited	Uncontrolled
Effectiveness of monitoring and mitigation plans	Proven effective	Untested or unlikely to be effective	

* Position on scale is only qualitative or semi-quantitative. The importance of position on one scale may be contingent on another scale. The importance of particular scales will vary with different cases.

latory approach because of the diversity of products that can be developed and the complexity of predicting their ecological fate. We expect that workable guidelines, to include categories of organisms requiring minimal screening and review, as well as those requiring more intensive review, will be developed after experience is gained from field experiments and from research that is stimulated by ecological risk assessment. These guidelines should be based upon the types of attributes of transgenic organisms and environments described in Table 1. The regulatory system should

remain flexible so that it can easily accommodate new information.

Product vs. process.—Current regulatory policy is focused on organisms produced by recombinant DNA technology (Office of Science and Technology Policy 1986). For the reasons discussed in the Scientific Issues section, **the phenotype of the transgenic organism, not the technique used to produce it, is the appropriate focus of ecological risk assessment and regulatory oversight.** Nonetheless, because many novel combinations of properties can be achieved only by molecular and cel-

lular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques.

Coding vs. non-coding regions.—Under current government policy, well-characterized, non-coding regulatory regions of the genome, regardless of their source, are assumed to pose no higher risk than does the unaltered recipient organism (Office of Science and Technology Policy 1986). Regulatory regions of the genome, however, serve to control the level and timing of the production of gene products, in some cases turning production on or off entirely. Thus, ecologically important aspects of the phenotype, such as substrate utilization, may be altered when a non-coding regulatory region is inserted (Colwell et al. 1987). In fact, an important and increasingly dominant theory of the origin of evolutionary novelties and higher taxa is that changes in regulatory regions are more important than changes in regions that code for gene products in producing these major evolutionary shifts (King and Wilson 1975, Paigen 1986). In addition, random insertion of promoters into the chromosome can cause expression of more than the inserted construct, including previously silent genes. Thus, **non-coding regulatory regions should not be exempted from ecological risk analysis and regulatory oversight.**

Categorization by lists of taxa and vectors.—Taxonomic lists of organisms and vector type are used by some regulatory agencies in determining the level of review required for a proposed introduction (e.g., National Institutes of Health 1986). Such categorization of safety by taxonomic listing is sound in some cases, but not in others. The biology of most crop plants is well understood, whereas the ecological characteristics of many bacteria are not, and the ecological characterization of vectors is nonexistent. A belief that any given gene exchange demonstrable in the laboratory is commonplace in nature is an hypothesis that has yet to be confirmed or rejected, and thus should not be the cornerstone of regulatory policy. **Lists of taxa and vectors categorized for environmental safety are only appropriate when substantiated by an ecological data base for that organism, including data on the attributes listed in Table 1.**

Commercial vs. noncommercial research.—The current regulatory framework partitions regulatory oversight according to funding source and existing statutory authority (Office of Science and Technology Policy 1986). One particularly troublesome aspect of this practice is the separation of commercial (privately funded) research from noncommercial research. From a scientific point of view, the risk from the introduction of a particular transgenic organism is the same whether the introduction is made by a scientist from the private

sector or a university professor. Scientific concerns should guide oversight of planned introductions into the environment regardless of funding source. **We urge that ecological risk assessment be sound and equal for both commercial and noncommercial research.**

Development of regulatory oversight

The first deliberate environmental introductions of genetically engineered organisms have been made using organisms altered in ways that present minimal ecological risk (e.g., Kluepfel et al. 1988, Lindow et al. 1988). Maintaining this approach for now allows time for the development of appropriate ecological risk assessment methods for future use, possibly including a set of categories and risk factors such as we have outlined in Table 1. We are concerned, however, that because the first introductions are especially likely to be innocuous, there may be a tendency to overgeneralize from these examples. **The absence of problems at an early stage suggests that the screening mechanisms are working correctly, but should not be interpreted to mean that the introduction of all genetically engineered organisms is inherently safe.** The appropriate use of early experience from field introductions is to establish principles and refine the regulatory structure in order to balance true risk with the extent of review.

We encourage the gradual development of biotechnology products and of regulatory oversight. As the biotechnology industry develops, continuing regulatory oversight, as well as long-term research and monitoring, are necessary for responsible risk management.

Consistency of regulation over political boundaries

Ecological effects and the geographic ranges of organisms transcend political boundaries; therefore, we consider it essential to promote and achieve international coordination of risk assessment and regulation of biotechnology. Because the potential hazards of engineered organisms are often environment-dependent, and ecosystems and biotas vary geographically and climatically, an organism that is safe in one country (or one state) is not necessarily safe in another. Thus both the commercial import and export and the inadvertent dissemination of engineered organisms or their genes across political boundaries present special concerns that require cooperation and coordination. Special consideration must be given to the protection of rare genetic resources, such as the wild ancestors of domesticated species, and threatened gene pools of other wild species. **We urge local, state, national and international cooperation in regulation, risk assessment, and risk management of the ecological effects of the introduction of genetically engineered organisms.**

Interdisciplinary research and education

There is a pressing need for interdisciplinary collaboration between molecular biologists, cell biologists, physiologists, ecologists, evolutionary biologists, and systematists in the development and environmental introduction of genetically engineered organisms. The new intellectual challenges and technical capabilities arising from this prospect suggest exciting research possibilities in key areas of ecology and evolutionary biology. Examples of such research include the study of population structure, community structure, and genetic diversity; gene flow, selection, speciation, hybridization, and other evolutionary processes; microbial ecology and evolution; the effects of limiting factors on abundance and distribution; the susceptibility of communities to invasion and changes in community structure; and the biological mechanisms of ecosystem processes. In turn, as we have stressed throughout this report, molecular and cell biologists stand to profit from the perspective and expertise of ecologists and evolutionary biologists in attaining both effectiveness and safety in the development of new products of biotechnology.

Interdisciplinary discourse is never easy to achieve and maintain. Cross-disciplinary conferences, graduate training programs, and one-on-one collaboration will be needed. The Ecological Society of America is ready to work with other scientific organizations and with regulatory agencies in promoting interdisciplinary research, education, and the incorporation of scientific information into the policy-making process. **For society to realize the full benefits of biotechnology, interdisciplinary research and graduate training programs are needed to expand the expertise of the scientific community at large.**

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MEMBERS OF THE WORKSHOP COMMITTEE:

INTRODUCTION OF GENETICALLY ENGINEERED ORGANISMS

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NOTE ADDED IN PROOF:

A completed table of examples of levels of safety concern for unmodified organisms as developed by members of the Working Group is included as Attachment 4.

DRAFT

Classifying Unmodified Organisms

A basic premise of the guidelines is that the conditions for safety in conducting research with a genetically modified organism are relative to attributes of the unmodified organism. These attributes include, but are not limited to, ability to survive and persist; ecological relationships with other organisms, potential to cause alteration of the genetic structure of natural or managed populations of the organism, and potential for control.

The ecological attributes of any organism cannot be assessed without an understanding of the environment in which it lives. Therefore, in assessing the safety condition of an unmodified organism to be used in genetic experiments, the organism's attributes must be judged relative to the environment accessible to it if it escaped from confinement at the specific test site.

If the ecological attributes of an unmodified organism in a specified accessible environment are very well understood and it can be determined that these attributes preclude the organism causing significant adverse effects, even after significant genetic modification, the organism can be assigned to the lowest level of safety concern of a 1. Attributes that could contribute to the classification of an unmodified organism as 1 include but are not limited to inability to survive in the accessible environment, lack of evolutionary potential to become a harmful organism, existence of practical techniques to totally prevent escape of viable individuals, existence of practical techniques to recapture or kill any escaped individuals before adverse effects occur, inability to exchange genetic information with native populations of its own species or other species.

On the opposite extreme, we may have an understanding of an organism's attributes indicating that no feasible types of confinement would allow the safe conduct of an experiment at a specified test site. Such organisms would be placed at concern level 5. Attributes contributing to such high concern include, but are not limited, to high capacity to survive and proliferate in the accessible environment, history of adverse effects caused by the organism in other environments, non-indigenous status in the accessible environment, lack of practical and adequate confinement techniques, lack of practical techniques for recapturing or killing escaped individuals.

While the placement of certain unmodified organisms in categories of very high or very low concern will be straightforward to most scientists, it will be difficult to pinpoint the level of concern for other organisms. We offer the following procedure as a generic tool for aiding the process of classifying unmodified organisms to levels of concern between 1 and 5. By gathering the types of information outlined below as ACTIONS I and II, a scientist will be in a reasonable position to evaluate the relative importance of specific attributes (ACTION III) and then explain his/her rationale for placing an unmodified organism at a specified level of concern (ACTION IV). We also offer a number of examples of specific cases (Appendix XXX) as further guidance.

ACTION I. Describe the accessible environment.

(give details)

ACTION II. Describe relevant aspects of the organism in the accessible environment:

1. Pest/pathogen status, low concern \longleftrightarrow high concern.
 - a) Assess the possible adverse effects of your organism on the accessible environment due to pest status or pathogenicity. Adverse effects may include but not be limited to lowered productivity of economically important organisms, damage or destruction of natural habitats, or adverse effects on human health. Extent of adverse effects may be judged relative to the existing conditions in the accessible environment.
 - b) Document the potential for exchange of genetic information between your organism and pest/pathogens in the accessible environment. Although your organism may not be a pest/pathogen, the opportunity may exist that it may become one by the occurrence of such an event.
 - c) Document ecological characteristics of your organism that allow or inhibit it from becoming a pest or increasing in pest/pathogen status. For example, if your organism and its relatives were restricted to a narrow set of ecological conditions (niche), potential to broaden that niche and become a pest is expected to be low.
 - d) Other.
2. Assess the potential of the unmodified organism to establish itself in the accessible environment. The information needed to judge this concern can be gathered by addressing the following:

Low concern \longleftrightarrow high concern

- a) Known mechanisms of survival or persistence in the natural environment; include natural biotic and/or abiotic control.
- b) Known mechanisms of dissemination.
- c) Effects of population size of the organism on establishment.
- d) How competitively aggressive is the organism in the accessible environment?

3. Ecological relationships with other organisms.

Low concern \longleftrightarrow high concern

The types of effects and interactions an unmodified organism has on and with its environment are related to its place in the community structure. The P.I. must be able to determine the extent to which the unmodified organism relates to others in its environment and how important it is to that community's structure. This will enable the P.I. to get a sense for whether any unforeseen changes in the modified organism could adversely affect the environment.

In order to determine whether or not the P.I. should rank ecological interrelationships between the unmodified organism and other organisms as being of low concern or high concern, in the proposed experiment, the following questions need to be answered:

- a) What is the importance of the unmodified organism to the structure of the community? In other words, how does the unmodified organism contribute to the structure of the community and how important is it. Some of the attributes that will help answer this question include:
 - Is it involved in any critical ecosystem functions? e.g., nitrogen fixation, inorganic nutrient uptake, key food chain component, critical habitat for key species, etc.
 - Is that involvement indirect or direct?
 - Can other organisms already present in the ecosystem fulfill its function?
- b) Ecological specificity. Does the unmodified organism occupy a broad or narrow range of differing habitats and environments. This question tells us something about the range of interactions an organism may have with other species.
- c) Extent of geographic range. Is the geographic range small or large? Are there changes that could occur in the organism to broaden or narrow its geographic range?
- d) Habit. Is the unmodified organism free-living, mutualistic, pathogenic, parasitic, or symbiotic? How does its habit relate to possible effects on the environment should it escape from confinement? Given the context of the experiment, will the habit of the unmodified organism allow you to easily stop its spread or prevent it from having adverse effects on the environment?
 - Note that the habit (free-living to parasitic) may tell you something about ecological specificity of the organism and its importance in community structure.
- e) Other.

4. Potential for introduction of genetic change in natural or managed populations.

The potential for genetic change in natural or managed populations resulting from the release of a genetically modified organism depends upon the genetic properties of the unmodified organism and the genetic structure of the populations that could be affected. The P.I. should consider the intrinsic genetic stability of the genome, such as the ability of organisms to incorporate exogenous DNA, the presence of active transposable elements, presence of active viral elements that interact with the normal genome or mutations that have resulted in an unusual genotype. The P.I. should also consider the size of the

natural or managed interbreeding population, the degree of genetic diversity in the population, and the potential for genetic exchange between an individual "released" organism and an organism in the natural population.

These factors should be evaluated with regard to the potential, high or low, for a released organism to influence the genetic structure of a natural or managed population. An unmodified organism with a high potential to change the genetic structure of a population could have, for example, high genetic instability, unusual autonomously replicating DNA elements, high potential to interbreed with natural or managed populations, where the diversity is low and the population size is small. An organism with normal genetic stability, no unusual DNA elements, limited potential to interbreed, where the populations are large and diverse genetically, would have a low potential to affect the genetic structure of that population.

5. Potential for control: The P.I. should be asked to provide information on:

- a) Prior experiments (lab and field) and demonstrated control (biological, environmental, physical, etc.) of the unmodified organism.
- b) The monitoring methods: are they routine -- how precise or accurate?
- c) How to handle an inadvertent release of the "test" organism.
- d) Other.

ACTION III. Evaluate relative importance of specific attributes. Carefully evaluate all of the above attributes and explain why some are more or less important in the context of the specific test you are planning.

ACTION IV. Choose safety condition of unmodified organism. Give your rationale for assigning your organism to safety conditions 1 through 5, which are described above.

WORKSHEET FOR ASSESSING RELEVANT ATTRIBUTES
OF UNMODIFIED ORGANISMS

Imported Fire Ant - Ann Sorensen

Imported fire ant, Solenopsis invicta Buren, single queen form. Study site: Athens, Georgia (the imported fire ant is currently established in 10 states including Georgia; the single queen form appears to be more common than the multiple queen form, but researchers feel the multiple queen colonies may be more difficult to control).

Accessible environment: If spread of fire ant colonies are limited to natural means, most newly mated fire ants will establish within 1 mile of their mother colony following a mating flight. Distances of up to 12 miles have been recorded following mating flights (Lofgren et al. 1975). In addition, fire ant mounds can relocate by either moving their entire colony or by colony budding, usually in the immediate area (Greenberg et al. 1985). Man-aided dispersal of fire ants (by transporting nursery stock or by inadvertent transport of newly mated queens) can extend the range of any colony into any area of the U.S. where weather and moisture conditions will permit their successful establishment (Tschinkel 1982, Vinson and Sorensen 1986).

1. Pest/pathogen status: 5

- a) Assess effects on accessible environment. The fire ant is primarily a pest of humans with its defensive behavior and multiple stings. It can also damage agricultural crops, either directly or indirectly (by tending aphids). Its mounds can damage harvesting

equipment and active colonies can discourage hand labor in fields. In addition, fire ants can harm farm animals (Lofgren 1986).

- b) Potential for exchange of genetic information. The fire ant can interbreed with at least one other species of fire ant in the U.S. (Solenopsis richteri) (Diffie et al. 1988). There may be other cases of hybrid populations in South America where this ant originates (Wilson 1952, J.C. Traeger, unpublished data). Some researchers hypothesize that the invicta-richteri hybrid is more cold tolerant (Wilson 1952).
- c) Ecological characteristics which affect pest status. The fire ant is a weedy species, preferring disturbed habitats (Tschinkel 1982). It is omnivorous and reproductively prolific (Lofgren 1986, Tschinkel 1982). It can withstand prolonged periods of flooding by forming rafts and carrying queens to safety (Anonymous 1958, Vinson and Sorensen 1986). These attributes could allow it to increase in pest status.
- d) Other. -

2. Ability to establish: 4

- a) Known mechanisms of survival. Only known mechanism to survive prolonged freezing temperatures is to construct mounds near sources of heat (Vinson and Sorensen 1986).

- b) Known mechanisms of dissemination. Natural flight of reproductive forms is 1/4 to 1 mile; fire ants can also spread by colony budding. Reproductives can "hitch-hike" on vehicles or in potted nursery stock. Fire ants can also be flooded out and reestablish downstream (Vinson and Sorensen 1986, Lofgren et al. 1975).
- c) Effects of population size. One newly mated queen can successfully found a colony (given the right conditions), which can produce thousands of reproductives when mature (Lofgren et al. 1975).
- d) Aggressiveness. The fire ant has shown itself to be very competitive with other ants, including other fire ant colonies (Anonymous 1958, Lofgren et al. 1975).
- e) Other. -

3. Ecological interrelationships: 3

- a) Importance to community. Fire ants tend to become a dominant predator where they establish. The diversity of the community diminishes accordingly. Other ant species seem especially vulnerable (Vander Meer 1987, Porter and Savignano 1989, Wilson et al. 1971).
- b) Niche specificity. Fire ants occupy a broader niche than many ant species because of their generalized feeding habits (Lofgren et al. 1975, Tschinkel 1982).

- c) Extent of geographic range. The potential range of the fire ant is limited by temperature (cold) and availability of water (Lofgren et al. 1975, Tschinkel 1982).
 - d) Habitat. Fire ants are free-living and predatory. They are not unduly restricted by either their nesting or feeding habits.
 - e) Other. -
4. Potential for genetic change (data not adequate, therefore assign a higher number): 4
- a) Interbreeding population size. We have no adequate data. The original introduction into the U.S. was probably small, so the genetic base may also be limited. Multiple queens may be inseminated inside the mound by mates without a mating flight (Glancey and Lofgren 1988, L. Greenberg, unpublished data).
 - b) Genetic stability/mutagenicity. No adequate data; diploid sterile males are produced by both forms (single and multiple queens). (Ross and Fletcher 1986).
 - c) Potential for genetic exchange. Solenopsis invicta can interbreed with S. richteri (see 1b).
 - d) Degree of genetic diversity. Data is currently being gathered (K.

Ross, personal communication) (see 4a).

e) Other. -

5. Potential for monitoring and control: 4

- a) History of use and control. The track record on control of fire ants is extensive but poor; individual mound control is relatively easy; area-wide control is difficult; eradication is impossible (Lofgren 1986, Banks et al. 1978, Buren et al. 1978).
- b) Accepted monitoring methods. Newly established colonies are nearly impossible to locate; monitoring is usually done with baits or pitfall traps (Banks et al. 1978).
- c) Accepted methods of control. Individual mound treatments are reliable; a combination of baits and mound treatments could eliminate all ants from a test site providing no new queens migrated in to start new colonies (Vinson and Sorensen 1986).
- d) Control of inadvertent release. Control of inadvertent escapes is difficult in that it would be extremely hard to locate ants. Unfortunately, colonies will often move in response to disturbance (Vinson and Sorensen 1986).

Evaluation of Safety Condition:

The imported fire ant can be beneficial in limited situations (for example, as primary predators in sugar cane fields and on lone star ticks), but most people feel they are both an economic and nuisance problem. In addition, most areas which are invaded by fire ants seem to suffer from decreased species diversity as a result of their establishment. However, the fire ant can be controlled if treatments are made periodically and the area proposed for the research (Athens, Ga.) already supports a fire ant population. For these reasons, the unmodified organism rates a "4" rather than a "5."

UNMODIFIED FIRE ANT = 4

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Africanized Honey Bee - Ann Sorensen

AHB: African honey bee - Apis mellifera scutellata. Proposed site of research: Harlingen, Texas (semi-tropical, intensive agricultural production with many crops dependent on pollination by European honey bees (EHB). Large wild populations of European bees, important overwintering site for northern state beekeepers).

Accessible environment: It has been estimated that Africanized honey bees can travel up to 200-300 miles per year through natural colony swarming (Taylor 1985). In addition, colonies could inadvertently be hauled to new locations by beekeepers or survive on shipments of equipment or other items from infested areas (Gary et al. 1985). This means that the range of the Africanized honey bee is limited only by temperature extremes, and researchers have not totally established what those temperature limitations are. The accessible environment, currently, would include anywhere from one-third to all of the U.S.

1. Pest/pathogen status: 4

- a) Assess effects on accessible environment. The AHB tend to be very defensive, and their behavior can be unpredictable. Because of these characteristics and their propensity to swarm, they may adversely affect humans and pollination services (Gary et al. 1985, Fletcher 1978).
- b) Potential for exchange of genetic information. AHB can exchange genetic information with domestic bees. It appears now that hybrids do

not survive as well in the environment as either of their parent strains (Hall and Muralidharan 1989, Smith et al. 1989, Page 1989).

- c) Ecological characteristics which affect pest status. Breeding in South Africa and South America has demonstrated a potential to lessen defensive characteristics through selection (Fletcher 1978, Taylor 1985, Gary et al. 1985).
- d) Other. Effects of temperature - AHB may be less defensive in more temperate climates (Otis 1982, Weaver 1976).

2. Ability to establish: 5

- a) Known mechanisms of survival. AHB can nest anywhere; they seem to be able to overwinter in areas with snowfall, but cold hardiness is a weak point (Kerr et al. 1980, Katznelson 1971).
- b) Known mechanisms of dissemination. AHB's are strong flier; they swarm frequently and their reproductive potential is high. They have been moving at a rate of about 200 miles per year since their introduction into Brazil in the 1950s. (Otis 1982, Taylor 1985, Hall and Muralidharan 1989).
- c) Effects of population size. Even one colony of AHB can produce many swarms (Taylor 1985, Otis 1982).

d) Aggressiveness. AHB tend to be very aggressive. They seem to compete well with other bees and can take over EHB hives (Taylor 1985, Otis 1982, Rinderer 1985).

e) Other -

3. Ecological interrelationships: 3

a) Importance to community. AHB is an important pollinator where it has established, but it is replaceable by other subspecies.

b) Niche specificity. Like EHBs, the AHB needs access to water and pollen and nectar sources (Fletcher 1978, Taylor 1985).

c) Extent of geographic range. AHB is currently found in South Africa, parts of South America, Central America and Mexico. The potential to expand its range is based on its overwintering capabilities; most scientists predict it will establish and drive out EHB in Rio Grande Valley. (Taylor 1985, Taylor et al. 1988).

d) Habitat. -

e) Other. -

4. Potential for genetic change: 4

- a) Interbreeding population size. AHB can interchange genetic material with all other Apis mellifera (Otis 1982, Taylor 1985, Hall and Muralidharan 1989).
- b) Genetic stability/mutagenicity. With selective breeding, AHBs seem to have enough genetic variability to either cause more problems or less.
- c) Potential for genetic exchange. AHBs will interbreed with EHB, although current information indicates that the occurrence of hybrids is low (see e).
- d) Degree of genetic diversity. AHBs in Mexico can essentially be traced back to 26 queens from South Africa (Page 1989).
- e) Other. When it interbreeds with EHB, the AHB genome dominates (Hall and Muralidharan 1989, Smith et al. 1989, Page 1989).

5. Potential for monitoring and control: 4

- a) History of use and control. We have a long history of use of AHB in South Africa and 40 years in South America. The control of wild swarms is difficult and not absolute (Taylor 1985, Gary et al. 1985).

- b) Accepted monitoring methods. We have developed adequate monitoring techniques, but as of yet they are not rapid or cheap (Gary et al. 1985, Rinderer et al. 1986).
- c) Accepted methods of control. Currently, only methods of control for individual swarms exist. Many pesticides are lethal to honey bees and individual swarms can be lured into bait traps using pheromone lures (Taylor 1985, Gary et al. 1985).
- d) Control of inadvertent release. We have no blanket control for AHB. They are too closely related to EHB, and any area-wide suppression of AHB could adversely affect EHB as well.

Evaluation of Safety Condition:

Ecological disturbances seem to be intense for the first few years. Eventually AHB establishes, becomes the dominant honey bee and beekeepers can learn to successfully manage them and eventually domesticate (i.e., gentle) them. AHB will establish in Harlingen, Texas, regardless of whether humans put them there inadvertently or not.

UNMODIFIED AHB = 4

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Pseudomonas fluorescens 2-79 - Rodney Bothast

Pseudomonas fluorescens 2-79, originally isolated from wheat plant roots, will be genetically modified for increased phenazine-1-carboxylic acid production and tested as a seed treatment to protect wheat from the disease, "take-all." Field plots will be established at Washington State Univ., Pullman.

Accessible environment: The accessible environment would include soils in the test plots and in close proximity to the test plot.

1. Pest/pathogen status: 2

- a) Assess effects on accessible environment. The unmodified organism has no known adverse effects on man or the environment and has a history of biocontrol use (Weller and Cook 1983). This organism has a nonpathogenic relationship with the root tissue of wheat plants (Weller 1983). Other strains of the species have been isolated from diseased plants, spoiled food, and clinical specimens (Polleroni 1984).
- b) Potential for exchange of genetic information. The unmodified organism is a normal soil bacterium that could possibly exchange genetic material with other soil bacteria.
- c) Ecological characteristics which affect pest status. The organism is ubiquitous (soil and water) (Weller et al. 1985), and experiments have shown that this strain decreases in numbers with time in the soil (Weller 1984).

2. Ability to establish: 2

- a) Known mechanisms of survival. The organism is common in soil and water, and no spores are formed (Palleroni 1984).
- b) Known mechanisms of dissemination. Normal dissemination methods apply (e.g., water, wind, insects, ect.). However, previous experiments show that this unmodified organism moves very little in the soil (Howie et al. 1985).
- c) Row-to-row movement does not occur, and migration from the plant root is very slight - 6 cm. This organism competes well with indigenous bacteria in soil for approximately 48 days, and then declines in number (i.e., poor survival) (Weller and Cook 1983).
- d) Aggressiveness. -
- e) Other. -

3. Ecological interrelationships: 1

- a) Importance to community. The community of plant inhabitants (P. fluorescens 2-79, one of many bacterial strains involved) presents a natural living barrier against disease. Protection results from competition for nutrients, inhibitory substances produced by the colonist (siderophores and/or antibiotics), hyperparasitism, etc. The unmodified organism alone does not improve wheat yield. However, this organism colonizes roots and provides better protection when the ratio

of the introduced strain (2-79) is high relative to indigenous bacteria (Weller and Cook 1983).

b) Niche specificity. Niche and geographical range of the unmodified organism is quite broad.

c) Extent of geographic range. See (b).

d) Habit. -

e) Other. -

4. Potential for genetic change: 2

a) Interbreeding population size. (See 1b above)

b) Genetic stability/mutagenicity. Could incorporate exogenous DNA, transposons, plasmids, etc., but is of limited potential to harm humans and the environment because of poor persistence.

c) Potential for genetic exchange. - Has potential for genetic exchange [see 1b above]

d) Degree of genetic diversity. -

e) Other. -

5. Potential for monitoring and control: 1

- a) History of use and control. Numerous laboratory and field experiments have been conducted with the unmodified organism (Weller and Cook 1983). Genetically marked strains would be desirable (e.g., antibiotic resistance).
- b) Accepted monitoring methods. Routine differential plating techniques have been used to isolate and enumerate the test organism (Howie et al. 1985).
- c) Accepted methods of control. The organism moves very little and does not survive in high numbers; consequently, no control would be required (Howie et al. 1985).
- d) Control of inadvertent release. -

Evaluation of safety condition:

As Principal Investigator, I have subjectively integrated the information on the five attributes for P. fluroescens 2-79 and recommend that the unmodified organism be placed in Safety Category 2. Of most concern to man and the environment are its pest/pathogen status and potential for genetic change.

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Butyrivibrio fibrisolvens - R. B. Hespell

A genetically engineered, hyper-hemicellulose, digesting strain of Butyrivibrio fibrisolvens will be tested in ruminants for increased performance (milk production in cows, weight gain in steers). The study will involve 12 ruminant animals housed in a facility, physically isolated from other animals, on a farm 12 miles from Ames, Iowa.

Accessible environment: The accessible environment includes the test animals and, for a limited time, the animal facilities.

1. Pest pathogen status: 1

- a) Assess effects on accessible environment. The unmodified organism is a natural ruminal bacterium and is also present in biogas digestors (Sewell et al. 1988). There are no known reports of toxin production by this organism or involvement in animal or human disease.
- b) Potential for exchange of genetic information. Not known, but organisms have plasmids (Mann et al. 1986).
- c) Ecological characteristics which affect pest status. The unmodified organism is an obligate anaerobe with a primary ecological niche in gut animals (Hespell and Bryant 1981, Lewis and Dehority 1985).
- d) Other. -

2. Ability to establish - 1

- a) Known mechanisms of survival. The organism does not survive or survives poorly in aerobic environments or low-organic environments (see 1C).
- b) Known mechanisms of dissemination. The organism is not known to form spores or resistant forms (Hespell and Bryant 1981).
- c) Effects of population size. It is a predominant species in the mammalian gut (Bryant and Small 1956, Lewis and Dehoity 1985).
- d) Aggressiveness. It competes well with other microbial species in the gut of ruminants, swine, etc.
- e) Other. - The organism is sensitive to many antibiotics.

3. Ecological interrelationships: 1

- a) Importance to community. The importance of the unmodified organism to the rumen is high, but other ruminal bacteria can fulfill the function of hemicellulose degradation (Hespell et al. 1987, Dehority 1966).
- b) Niche specificity. Broad: xylans, pectin, starches, proteins, lipids (Hespell and Bryant 1981).
- c) Extent of geographic range. Worldwide list of strain isolations.

d) Habit. -

e) Other. -

4. Potential for genetic change: 1

a) Interbreeding population size. The unmodified organism has limited potential for genetic change. There is little information in this area, but lab strains are stable for over 20-30 years.

b) Genetic stability/mutagenicity. There are no unusual DNA elements, but the organism probably can take up DNA/transposons/conjugative plasmids, etc. (Hespell, unpublished data). Genes can be cloned (Sewell et al. 1989).

c) Potential for genetic exchange. -

d) Degree of genetic diversity. -

e) Other. -

5. Potential for monitoring and control: 1

a) History of use and control. None.

b) Accepted monitoring methods. The potential for monitoring is very high. The genetically modified organism would have a unique chromosomal DNA sequence, as well as the unique 16S RNA sequence found

in the unmodified organism. Easy cultivation using anaerobic methods (Hespell and Bryant 1981).

- c) Accepted methods of control. Control should be good. The organism is sensitive to oxygen and antibiotics (see 2a).
- d) Control of inadvertent release. A genetically modified strain engineered (e.g., suicide system, specific nutrient requirement) to have limited survival outside rumen ecosystem would be desirable.

Evaluation of safety condition:

As Principal Investigator, I would recommend that the unmodified organism be placed in Safety Category 1 because of its natural habitat and its sensitivity to oxygen.

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Drosophila melanogaster, wild type laboratory strains - Ron Sederoff

Accessible environment: Drosophila melanogaster is found over wide areas of the world, in temperate and tropical regions of many continents. It is widely associated with, but is not restricted to, areas with human populations (Patterson and Stone 1952).

1. Pest/pathogen status:

- a) Assess effects on accessible environment. Wild type D. melanogaster have been cultured in the laboratory for over 80 years representing about 2000 generations beginning, with the work of Castle, followed by Morgan after the turn of the century (Carlson 1966). There are no known adverse effects on human health, agriculture, or to accessible environments from these organisms. They are considered as nonpests and nonpathogens.
- b) Potential for exchange of genetic information. None of the known natural organisms with which laboratory wild type strains of Drosophila melanogaster can interbreed are pests or pathogens. This organism can only exchange information with wild members of its own species (Lemunier et al. 1986). In the laboratory, it can form sterile hybrids with a small number of sibling species (Lemunier et al. 1986). Therefore, D. melanogaster is unlikely to form any hybrids in the wild. None of these sibling species are pests or pathogens.

c) Ecological characteristics which affect pest status. The organism has a cosmopolitan ecological niche which is closely associated with human populations (Patterson and Stone 1952, Parsons and Stanley 1981).

d) Other. -

2. Ability to establish:

a) Known mechanisms of survival. The potential of laboratory strains to become established is significant. The survival of wild type organisms is possible in both temperate and tropical environments.

b) Known mechanisms of dissemination. Dispersal is wide and is accomplished by flight of the organism (Taylor and Powell 1983).

c) Effects of population size. The laboratory organism is unlikely to be more competitive than the natural organism in the natural environment. Many generations of growth under laboratory conditions may have resulted in reduced ability to survive in the natural environment.

d) Aggressiveness. -

e) Other. -

3. Ecological interrelations with other organisms:

- a) Importance to community. The role of D. melanogaster in community structure is likely to be small. It is a free-living, nonparasitic organism whose populations are at low levels compared to many insect pests. It is not known to be essential for any critical ecosystem function.
- b) Niche specificity. It has a somewhat restricted ecological niche. The geographic range is broad, but it is closely associated with human populations.
- c) Extent of geographic range. The geographic range is broad, but it is closely associated with human populations.
- d) Aggressiveness. -
- e) Other. -

4. Potential for genetic change:

- a) Interbreeding population size. -
- b) Genetic stability/mutagenicity. The organism has no unusual properties with respect to genetic instability, uptake of exogenous DNA or the presence of viral elements (Lindsley and Grell 1967, Ashburner and Thompson 1978). The level of diversity in natural populations is relatively high (Tracey and Ayala 1974), and the release of an

unmodified organism into the breeding population would be unlikely to have any effect on the genetic structure of the population.

- c) Potential for genetic exchange. The release of an unmodified organism into the breeding population would be unlikely to have any effect on the genetic structure of the population.
- d) Degree of genetic diversity. The level of diversity in natural populations is relatively high (Tracey and Ayala 1974).
- e) Other. -

5. Potential for monitoring and control:

- a) History of use and control. It is difficult to contain D. melanogaster under any laboratory conditions, but a high degree of containment can be obtained using specific genetic modifications that would preclude the transfer of genetic information to organisms in the environment.
- b) Accepted monitoring methods.
- c) Accepted methods of control. A combination of high level containment and genetic manipulation of the organism by conventional mutation and crosses would provide a high level of control. Alternatively, Drosophila are resistant to high levels of ionizing radiation that can produce sterility but are not lethal to the organism.

- d) Control of inadvertent release. The difficulty of preventing escaping organisms is a major problem for the release of genetically modified organisms.

Evaluation of safety condition:

Drosophila is an exceptionally safe organism with a long history of human contact, however, the difficulty of preventing escaping organisms is a major problem for the release of genetically modified organisms. Therefore, I would classify the unmodified organism in level 2. Experiments with potential for release or experiments involving deliberate release into the environment should use genotypes that have some factor or factors (e.g., sterility) that reasonably prevent transfer of DNA to the natural Drosophila populations.

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Brassica napus in northwestern United States - John Kemp

Accessible environment: The release of Brassica napus might be into the seed production area of Washington State. Given that this organism is stationary, inadvertent release involves the spread of pollen by insects and, therefore, cross fertilization of other plant species as well as indigenous could occur. Inadvertent cross pollination of product fields will make the production seed worthless. Inadvertent cross pollination of weeds will spread the engineered traits into the wild populations of the Washington production area.

1. Plant/pathogen status:

- a) B. napus is extensively grown as an oil seed crop in southern Canada (Vaughn et al. 1976, Williams 1985) and is becoming more important as an oil seed crop in the U.S. (Vaughn et al. 1976, Williams 1985). Wild strains of the species are often considered weeds (Wilkinson and Jaques 1979). This is especially true because of their ability to cross with cultivated varieties in the Northwest where a large portion of the elite vegetable seed is produced for the U.S. seed market. Other weedy brassicas found in the Northwest are B. nigra, B. juncea, B. kaber, and B. hirta.
- b) Potential for exchange of genetic information. - The potential for exchange between the various Brassica species is high in the Northwest

because Brassica is extensively grown for vegetable seed production and because there are many indigenous weeds (see 1c for more details).

- c) Ecological characteristics which affect pest status. - Cultivated Brassica species represent one of the largest and most diverse families of interrelated species and subspecies. Their diverse uses range from oils (Brassica napus), condiments B. juncea), vegetables (B. oleracea), to animal fodders (B. napus). Included within the broad family are many weedy, wild brassicas, e.g., B. nigra (black mustard), B. juncea (Indian mustard), B. hirta (white mustard), and B. kaber (wild mustard). B. napus will outcross with all of these species. Generally, Brassica species are pollinated by insects with little or no occurrence of wind pollination (McGregor).

- d) Other. -

2. Ability to establish:

- a) Known mechanisms of survival. Adapts, could persist as do its closely related weedy relatives (Wilkinson and Jaques 1979).
- b) Known mechanisms for dissimulation. Seed; not vegetatively propagated.
- c) Effects of population size. Unknown. The larger the field of Brassica the more likely insects will find it and spread its pollen.

d) Aggressiveness. Not known. We suspect it will propagate in an unmanaged ecosystem.

e) Other. -

3. Ecological interrelationships:

a) Importance to community. Northwest species have low independence (Williams 1985).

b) Niche specificity. The niche for B. napus is cultivated farm fields but its weedy relatives includes wastelands and roadsides.

c) Extent of geographic range.

d) Habit.

e) Other. -

4. Potential for genetic change:

a) Interbreeding population size.

b) Genetic stability/mutagenicity.

c) Potential for genetic exchange. B. napus has the potential for genetic exchange with other species in the Northwest (Williams 1985).

d) Degree of genetic diversity.

e) Other. -

5. Potential for monitoring and control:

- a) History of use and control. Good control. B. napus is a higher plant, thus it is easily recognized and, except as seed or pollen, individuals do not move.
- b) Accepted monitoring methods. Survey suspect areas and visual identification of plants using taxonomic keys.
- c) Accepted methods of control. There are good chemical controls available and spread can be completely eliminated by not allowing plants to flower or by controlling the insects that B. napus depends on for pollination (McGregor). It is a non-vegetatively propagated annual plant. However, B. napus can survive in an unmanaged ecosystem and can cross with many other species, thereby avoids detection in hybrid forms.
- d) Control of inadvertent release. Cultural and chemical control can be utilized. Cultural methods would include cultivation, mowing, and hand pulling of plants. Chemical methods would include herbicides and fumigants to control plants and seed, respectively. Examples of herbicides would include 2, 4-D, dicamba, glyphosate, bromoxynil. The choice of chemical control would depend on the crop or ecological

situation where the escape occurred. Weed scientists would be able to recommend control measures.

Evaluation of safety conditions:

Evaluation of relative importance of specific factors: the most important factor is that B. napus can cross with many vegetable and weedy species. Therefore, the overall characteristics of this unmodified organism are of moderate concern for the purpose of genetic engineering experiments. I would assign B. napus a safety condition in Washington State of 3 or 4.

References:

- Kearney, T. H. and R. H. Peebles. 1960. Arizona flora. Univ. Calif. Press. 1085 pp.
- McGregor, S. Insect role of cultivated crop plants. Agriculture Handbook #46, USDA publication.
- Vaughn, MacLeod and Jones. 1976. Biology of the Cruciferae. Academic Press, New York.
- Wilkinson and Jaques. 1979. How to know the weed, third edition. W. C. Brown Co., Dubuque, Iowa.
- Williams, P. H. 1985. Crucifer genetics cooperative, resource book. Univ. Wisconsin, Madison.

Brassica napus in New Mexico and Arizona - John Kemp

Accessible environment: The release of B. napus might be into the Mesilla Valley near Las Cruces, N.M. Given that this organism is stationary, inadvertent release involves the spread of pollen by insects. Since there are very few vegetable crops or weedy species with which B. napus could cross, inadvertent pollination would be rare.

1. Plant/pathogen status:

- a) Assess effects on accessible environment. In some areas of the U.S., B. napus is considered a weed, mostly because of its ability to cross with cultivated Brassica crops. In contrast to other areas of the U.S., the southwestern states of Arizona and New Mexico, where the release is proposed, have very few indigenous Brassica species and do not identify any as weeds. B. napus would not be a pest because there are few vegetable crops or weeds with which to could cross (Vaughn et al. 1976, Wilkinson and Jaques 1979, Williams 1985).
- b) Potential for exchange of genetic information. The potential for exchange between the various Brassica species is low in New Mexico because Brassica is not extensively grown as a vegetable crop and because there are very few indigenous weeds (see 1c for more details).
- c) Ecological characteristics which affect pest status. Cultivated brassicas represent one of the largest and most diverse family of

interrelated species and subspecies. Their diverse uses range from oils (Brassica napus), condiments (B. juncea), vegetables (B. oleracea), to animal fodders (B. napus). Included within the broad family are many weedy, wild brassicas, e.g., B. nigra (black mustard), B. juncea (indian mustard), B. hirta (white mustard), and B. kaber (wild mustard). B. napus will outcross with all of these species. Generally Brassica species are pollinated by insects with little or no occurrence of wind pollination (McGregor). But, the overriding consideration for the Southwest should be that indigenous plants with which it can cross are not present.

d) Other. -

2. Ability to establish: Low concern

- a) Known mechanisms of survival. Adapts, could persist as do its closely related weedy relatives (Wilkinson and Jaques 1979).
- b) Known mechanisms for dissimulation. Seed; not vegetatively propagated.
- c) Effects of population size. The effects of population size are unknown. The larger the field of Brassica, the more likely insects will find it and spread its pollen.
- d) Aggressiveness. The aggressiveness of B. napus is not known. It is suspected that it will propagate in an unmanaged ecosystem.

3. Ecological interrelationships: Low concern

- a) Importance to community. Low concern. New Mexico species have low independence (Kearney and Peebles 1960).
- b) Niche specificity. The niche for B. napus is cultivated farm fields, but its weedy relatives includes wastelands and roadsides.
- c) Extent of geographic range. The geographic range of Brassica is worldwide.
- d) Habitat. Same as 3c.
- e) Other. There are very few weedy Brassica species in New Mexico and very little cultivated Brassica (Kearney and Peebles 1960).

4. Potential for genetic change: Low concern

- a) Interbreeding population size. Potential for exchange is great, but there are so few Brassica species present that the concern is low.
- b) Genetic stability/mutagenicity. Unknown.
- c) Potential for genetic change. B. napus has the potential for genetic exchange with other species in New Mexico (Kearney and Peebles 1960), but the overriding consideration should be that indigenous plants with which it can cross are not present.
- d) Degree of genetic diversity. Very diverse.
- e) Other. -

5. Potential for monitoring and control: Low concern

- a) History of use and control. Good control. B. napus is a higher plant, thus it is easily recognized and, except as seed or pollen, individuals do not move.
- b) Accepted monitoring methods. Survey suspect areas and visual identification of plants using taxonomic key.
- c) Accepted methods of control. There are good chemical controls available and spread can be completely eliminated by not allowing plants to flower or by controlling the insects that B. napus depends on for pollination. It is a non-vegetatively propagated annual plant. However, B. napus can cross with many other species and can survive in an unmanaged ecosystem and can cross with many other species, thereby avoids detection in hybrid forms (Vaughn et al. 1976, Wilkinson and Jaques 1979, Williams 1985).
- d) Control of inadvertent release. Cultural and chemical control can be utilized. Cultural methods would include cultivation, mowing, and hand pulling of plants. Chemical methods would include herbicides and fumigants to control plants and seed, respectively. Examples of herbicides would include 2, 4-D, dicamba, glyphosate, bromoxynil. The choice of chemical control would depend on the crop or ecological situation where the escape occurred. Weed scientists would be able to recommend control measures.

Evaluation of safety conditions:

Evaluation of relative importance of specific factors: the most important factor is that B. napus can cross with many vegetable and weedy species. However, there is little Brassica grown in New Mexico and very few weedy Brassica. Therefore, the overall characteristics of this unmodified organism are of low concern for the purpose of genetic engineering experiments. I would assign B. napus a safety condition in New Mexico of 1.

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- Kearney, T. H. and R. H. Peebles. 1960. Arizona flora. Univ. California Press. 1085 pp.
- McGregor, S. Insect role of cultivated crop plants. Agric. Handbook #46, USDA Publ.
- Vaughn, MacLeod, and Jones. 1976. Biology of the Cruciferae. Academic Press, New York.
- Wilkinson and Jaques. 1979. How to know the weeds, third edition. W. C. Brown Co., Dubuque, Iowa.
- Williams, P. H. 1985. Crucifer genetics cooperative, resource book. Univ. Wisconsin, Madison.

Zea mays - John Kemp

Accessible environment: The intended release site for Zea mays is mid-Iowa, the center of the corn-growing area for the U.S. Given that this organism is stationary and can not exist outside a managed ecosystem, inadvertent release involves the spread of pollen by wind to adjacent farm fields. This would be of little concern since most corn is cross-pollinated; the cultural practices for controlling cross pollination are well known.

Cultivated Zea mays represents the most commonly grown crop plant in the U.S. It is also one of the most highly domesticated plants used in agriculture. There are no known wild relatives or weeds with which corn can cross. Furthermore, corn will not survive in an unmanaged ecosystem. Corn is wind pollinated, not insect pollinated, and the pollen does not survive long after dissemination. Corn is grown in managed ecosystems in all parts of the U.S.

1. Pest/pathogen status:

- a) Assess effects on accessible environment. Low concern in Midwest corn-production areas. Zea mays is not considered a pest and will not survive on its own. It is known that viable pollen can not be disseminated more than 1/4 mile. Therefore, with some consideration of nearby corn fields, the pest/pathogen status is of low concern.
- b) Potential for exchange of genetic information. See 1a and introduction to paragraph.

c) Ecological characteristics which affect pest status. See introduction paragraph.

d) Other. -

2. Ability to establish:

a) Z. mays is unable to establish in the Midwest. Corn will not survive in an unmanaged ecosystem.

b) Potential for exchange of genetic information. There are no known wild relatives or weeds with which corn can cross.

c) Corn is wind pollinated, not insect pollinated, and the pollen does not survive long after dissemination.

d) Other. -

3. Ecological interrelationships:

a) Importance to community. Low independence.

b) Niche specificity. Narrow - only persists in specifically cultivated fields.

c) Extent of geographic range. Almost worldwide.

d) Habit.

e) Other. -

4. Potential for genetic change:

a) Unable to exchange. Even if there was some pollination of an adjacent corn field, the progeny would not survive.

b) Genetic stability/mutagenicity.

c) Potential for genetic exchange. Very low, except with other plants in a managed ecosystem.

d) Degree of genetic diversity. Very narrow.

e) Other. -

5. Potential for monitoring and control:

a) History of use and control. Z. mays is the most commonly grown crop plant in the U.S. and one of the most highly domesticated plants used in agriculture. It is grown in managed ecosystems in all parts of the U.S.

b) Accepted monitoring methods. Corn is a higher plant, thus it is easily recognized and individuals do not move, except as seed and pollen.

c) Accepted methods of control. There are good chemical controls available.

- d) Control of inadvertent release. Cross pollination does not occur easily and individual progeny will not survive outside a managed ecosystem.

Evaluation of safety condition:

Since corn is so highly domesticated, grown so widely in the U.S., and has no known weedy relatives in the U.S., the safety category for unmodified Z. mays would be Category 1 for release experiments in the Midwest.

References:

Chapman, S. R. & L. P. Carter (eds.). Crop production principles and practices. 1976. W. H. Freedman & Co., San Francisco.

Delorit, R. J., et al. 1984. Crop production. Prentice-Hall Inc., New Jersey.

Aldrich, S. R., et al. 1975. Modern corn production. A & L Publications, Illinois.

Official seed certification handbook for New Mexico. Published by New Mexico Crop Improvement Assoc.

Soybean Mosaic Virus - Sue Tolin

Soybean mosaic virus - Virginia strain, G1 strain group.

Accessible environment: Field inoculations of soybean cultivars to test for susceptibility and resistance to virus infection will be conducted at Blacksburg, Virginia.

1. Pest/pathogen status: 3

- a) Assess effects on accessible environment. Soybean mosaic virus (SMV) is regarded as an important disease in some parts of the world. Effects of virus infection include reduced yield resulting from reduced pod set and seed development, and seeds often have a discoloration of the hilum that reduces their quality. The effect depends on the environmental conditions, cultivar, virus strain, and plant age when infected. There are some reports of yields being reduced as much as 50% in any one field, and as high as 93% in experimentally inoculated plants (Ford et al. 1989). In field tests done by inoculating every plant with this particular strain, average yield losses of 14% were measured (Roane et al. 1974).

Effects on other plants. The host range of SMV has been extensively tested (Edwardson 1974). Only 30 species of plants in 20 genera in the family Leguminosae are reported to be susceptible to SMV. One or more species in 12 additional genera of Leguminosae are not susceptible to

SMV (Boswell and Gibbs 1983). Soybean, Glycine max, is its only known natural host (Bos 1972). Three species of Chenopodium are the only nonlegume hosts (Galvez 1962, Bos 1972, Boswell and Gibbs 1983). Plants in 12 other families have been tested.

- b) Potential for exchange of genetic information. There are no known mechanisms for exchange of genetic information between monopartite, single-stranded [+] RNA viruses, or between these viruses and their plant hosts, or other pests or pathogens (Matthews 1981).
- c) Ecological characteristics which affect pest status. SMV is a pest of moderate consequence. More severe strains may develop, but the mechanism by which this occurs is not known (Cho and Goodman 1979).
- d) Other. -

2. Ability to establish: 1

- a) In order to survive, a plant virus must have a host plant in which it can multiply, an effective means of spreading to, and infecting fresh host plant, and a supply of suitable healthy host plants to which it can spread (Matthews 1981, p. 608). Many viruses have a weed or other host in the natural environment, and spread from such initial sources or foci of infection into or within a crop (Walkey 1985). Soybean is the only known host in the accessible environment. Soybeans are annual plants in this environment, and will be killed by frost and winter

temperatures. The virus persists in a certain percentage of soybean seeds produced on infected plants (Bowers and Goodman 1979). Upon germination, these plants may be infected with the virus. The percentage of infected plants is affected by the time of infection in relation to plant development, as well as by the cultivar and virus strain (Irwin and Goodman 1981).

- b) Known mechanisms of dissemination. SMV is known to be transmitted by at least 31 species of aphids in a nonpersistent manner (Irwin and Goodman 1981, Abney et al. 1976). In Illinois and in Virginia, transient alate aphids are wholly responsible for the field spread of SMV, with the timing and rate of spread a function of activity of transient aphids that land on soybean leaves, probe, move to new plants, and probe again (Irwin and Goodman 1981). The vector intensity and factors affecting transmission has been extensively studied in Illinois (Irwin and Ruesink 1986, Schultz et al. 1983, Schultz et al. 1985). A predictive model for SMV spread and subsequent effects on yield, based on initial inoculum of infected seeds and vector intensity, has been developed from data generated in field tests in Illinois and validated by field tests in four additional states: Georgia, Louisiana, Mississippi, and Virginia (Ruesink and Irwin 1986).
- c) Effects of population size. There is no effect on the size of the population of virus on establishment. The dilution end point of crude sap is 10^{-3} to 10^{-4} (Galvez 1962). Inoculations are made with a 10^{-1}

dilution to assure a high percentage of successful inoculations (Roane et al. 1980).

d) Aggressiveness. SMV will not move from the inoculated plants without the aid of its aphid vector (Bos 1972, Matthews 1981). No plants in the accessible environment, with the exception of soybeans in the experimental plot, are susceptible to SMV, i.e., act as a host for the virus. In Blacksburg, VA, there are no commercial soybean fields within over 100 miles. Other experimental plots are over 5 miles distance.

e) Other. -

3. Ecological interrelationships: 1

a) Importance to community. Plant viruses are not free-living organisms and are not known to have any importance to community structure. Furthermore, SMV is not known to naturally infect any plants other than soybean in the accessible environment. Glycine max is the only angiosperm species found to be infected naturally (Boswell and Gibbs 1983). Usually when viruses do infect wild plants, the effect is very little since destruction of its host leaves the virus without a mechanism of persistence in the environment.

b) Niche specificity. SMV occupies a very specific niche, since only soybeans are known to be naturally infected.

- c) Extent of geographic range. SMV is known to occur worldwide in soybeans wherever they are grown, because it is seed-borne and transported with the seed wherever they are shipped. It is believed to have originated in China where soybeans originated and wild relatives are present.
- d) Habit. Like all viruses, SMV is totally parasitic and dependent upon suitable host cells for its replication (Matthews 1981, p. 11).
- e) Other. -

4. Potential for genetic change: 2

- a) Interbreeding population size. The term 'interbreeding population size' is not appropriate for viruses. The following is revised accordingly. Genetic structure of the organism: single-stranded RNA, 10,000 bases, coding for 7-8 genes (Dougherty and Carrington 1988). Genome map of related viruses has been determined, and exact sequence known for some potyviruses. Partial sequence known for this strain (Gunzyuzlu et al. 1987).
- b) Genetic stability/mutagenicity. Soybeans are the only organisms in the accessible environment that would be expected to be affected, because only soybeans are expected to be infected. The potential for the virus to induce genetic change in soybeans is unknown and not expected.

- c) Potential for genetic exchange. There is a slight chance that mixed infection of soybeans with other viruses could lead to genetic exchange between viruses. Although multipartite plant viruses are known to interact and potentially result in new viruses or strains (van Vloten-Doting 1985), there is no demonstration of this occurring with potyviruses (Dougherty and Carrington 1988).
- d) Degree of genetic diversity. Strains of SMV that could be differentiated by reaction on a series of soybean cultivars has been described by Cho and Goodman (1979). The relationship of these strains to each other, as well as to other potyviruses is currently being examined (Tolin, unpublished, Shukla and Ward 1989). At least one strain of SMV, strain N, is quite different from the G1 strain from Virginia, and is potentially quite similar to another potyvirus (Yu et al. 1989). Recent sequence analyses demonstrating regions of identity in the coat protein genome between different potyviruses (Shukla and Ward 1989) suggest that genetic exchange could occur in the event of a mixed infection of a plant.
- e) Other. -

5. Potential for monitoring and control: 1

- a) History of use and control. Soybean mosaic virus has been introduced into field research for a number of years in several locations. In Virginia, inoculations have been made with an artist's air brush in the

same location as the proposed trial (Roane et al. 1974, 1983; Buss et al. 1989). Illinois has introduced various strains of SMV intentionally via infected seed and by mechanical inoculation; and found limited spread within the field (Irwin and Goodman 1981). In studies with a highly transmissible strain (G5), the farthest infection from the source was ca. 45 m along the vector of the prevailing wind. However, 25% of all spread occurred within 2 m of the source, and 95% were within 17 m of the source. Almost no infections were noted in the check or uninoculated plots (Irwin and Goodman 1981).

- b) Accepted monitoring methods. Infection in soybean is usually manifested by visible symptoms. ELISA tests are quite sensitive and accurate, provided antiserum used is specific for reaction with SMV (Lister 1978, Moore et al. 1982). If it is not, other potyviruses may be detected in the test because of similarities in coat protein structure (Shukla and Ward 1989).
- c) Accepted methods of control. An effective method for controlling SMV is to use SMV-free seeds. An acceptable level of less than 1 in 10,000 is predicted by the Ruesink and Irwin model (1989). Virus-free seeds are obtained by eliminating SMV-infected plants in seed fields, and testing seeds for the presence of SMV by ELISA (Lister 1978).

Control is also achieved by using soybean cultivars resistant to infection with SMV (Kiihl and Hartwig 1979, Roane et al. 1983). Resistance is known to be controlled by a single dominant gene, but

alleles at the same locus are known to condition susceptibility or resistance to various SMV strains (Buss et al. 1989). Reducing vector intensity will limit spread of SMV within a field, and can be accomplished by a number of approaches (Gunasinge et al. 1986, 1988; Halbert and Irwin 1981, Irwin and Goodman 1981). Insecticides are not effective because spread is by transient aphids (Irwin and Goodman 1981).

- d) Control of inadvertent release. If liquid inoculum is spilled and released in the field test site, there is no consequence since the virus is quickly inactivated in the absence of its host. The measured longevity in vitro is two to four days (Bos 1972, Boswell and Gibbs 1983, Galvez 1962). Aphid vectors would not be expected to feed on any spilled inoculum and acquire the virus.

Evaluation of safety conditions:

The safety analysis indicates SMV is Safety Condition 2. The virus is an important pathogen of a major crop. This particular strain does not cause extensive loss (14%) even if all plants are infected. Thus it does not warrant a higher classification than level 3. It has little ability to become established because there are no known natural hosts of the virus, and it thus is not known to have any impact on the natural ecosystem in which an introduction may be made. In the agroecosystem, previous introductions have documented that the virus has little potential to spread beyond the site of introduction, even in an area (Illinois) where soybeans are a major crop.

Control and monitoring methods are well established procedures, and have been demonstrated to be reliable. The virus may have some potential to change genetically, primarily because of the inherent variability between and among viruses in the potyvirus group and the potential instability of single-stranded RNA viral genomes.

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Transgenic cow experiment - Bos taurus - Phillip A. O'Berry

Accessible environment: The study site is the Texas A&M University Agricultural Research & Extension Center at Overton, Texas. It is a large, well-managed center with beef cattle production facilities that are typical of this semitropical region. The accessible environment is the Center and the ranches and farms immediately surrounding it. Bovine embryos from normal cattle will be collected, centrifuged, microinjected with potentially desirable foreign genes, fertilized, and implanted into recipient cows to produce superior transgenic cows (Pursel et al. 1989).

1. Pest/pathogen status: 1

- a) Assess effects on accessible environment. Cattle have low adverse affect on the accessible environment. They can damage agricultural crops if not confined.
- b) Potential for exchange for genetic information. Cattle can exchange genetic information with other bovidae, but are not likely to do so if confined.
- c) Ecological characteristics which affect pest status: Cattle are herbivorous range animals. They can survive in nature if feed and cover are adequate (Ensminger 1983).
- d) Other. -

2. Ability to establish: 1

- a) Known mechanisms of survival. Forage and reproduce in range, brush, swamp, and forest conditions.
- b) Known mechanisms of dissemination. Cattle range free, moving on foot to feed, water, and cover.
- c) Effects of population size. Cattle need proximity for estrus detection and breeding to occur.
- d) Aggressiveness. Cattle are generally not aggressive or territorial. They usually avoid human contact in free-ranging conditions.
- e) Other. -

3. Ecological interrelationships: 2

- a) Importance to community. Cattle are large herbivores with no major importance to their community.
- b) Niche specificity. Cattle occupy no specific crucial ecological niche.
- c) Extent of geographic range. Domesticated cattle are found in most areas of North America but are much less able to survive year-round untended in the wild in the northern half of the U.S. because of adverse winter weather conditions.

d) Habit. Capable of becoming free-living in ecosystems that provide adequate feed, water, and cover.

e) Other. -

4. Potential for genetic change: 2

a) Interbreeding population size. Although cattle have the potential to interbreed and the U.S. population size is large, in most areas where cattle are produced, they are sufficiently confined and managed to prevent unintended interbreeding.

b) Genetic stability/mutagenicity. Significant mutations do not occur with high frequency in the Bos taurus genome.

c) Potential for genetic change. Bos taurus has the potential to exchange genetic material with other bovidae.

d) Degree of genetic diversity. Evidence compiled from research on bovine blood groups indicates that a rather broad genetic base exists in the U.S. cattle herd (C. Stormont).

e) Other. -

5. Potential for monitoring and control: 1

a) History of use and control. Technology and methods are now available to confine, monitor, handle, and control domestic cattle.

- b) Accepted monitoring methods. Cattle are usually monitored by visual methods used on individually identified animals. Other more sophisticated methods utilizing electronic technology (e.g., transmitters, transponders, computers) are also available and effective.
- c) Accepted methods of control. Cattle are usually controlled by confinement and capture.
- d) Other. Method used is to round up.

Evaluation of safety condition:

Cattle are large herbivores that can be confined with considerable certainty, individually identified and monitored, do not survive in the wild in much of the U.S. and are not likely to encounter an untended interbreeding population. These are the main characteristics of Bos taurus that justify the assignment of safety condition of 1.0 (low concern).

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Feral pig - Sus species. Susceptibility of feral swine to Pseudorabies virus -
Phillip O'Berry

Accessible environment: The study site is the USDA-ARS National Animal Disease Center in Ames, Iowa. It is a large, high security facility in central Iowa. The accessible environment is the Center and the farms and countryside surrounding it. Feral pigs will be caught in Georgia, transported to the Center and exposed to Pseudorabies virus to determine their ability to serve as carriers of this virus.

1. Pest/pathogen status: 3

- a) Assess effects on accessible environment. Feral pigs are damaging to their environment. They are large omnivores with the capacity to damage cultivated crops and transmit diseases to domestic swine and humans. Columbus brought them to the West Indies, and DeSoto shipped them to Florida in 1539 (Wiser et al. 1986). The early settlers brought pigs with them as they moved westward. Large herds of pigs ran in the forest until fall when they were collected, fattened in corn fields, and slaughtered.
- b) Potential for exchange of genetic information. Potential for exchange of genetic information with other feral pigs is high if sufficient population and proximity exist. Potential for exchange with domestic pigs is possible but rather low because those pigs are usually well enough managed to preclude contact.

c) Ecological characteristics which affect pest status. Since the animals are feral, they must find a mate in the wild and adapt to a rather hostile environment. They must find feed year-round, and locate cover and fresh water. They adapt well to swamp, forest, and other rough and sparsely inhabited areas.

d) Other. -

2. Ability to establish: 3

a) Known mechanisms of survival. Feral pigs have undergone centuries of adaptation and have many attributes that aid their survival. They are tough, eat almost anything, and are active foragers.

b) Known mechanisms of dissemination. Feral pigs move on their own power in search of feed, water, and cover. Their movement has also been aided by humans who sometimes capture, move, and release them for hunting purposes.

c) Effects of population size. A critical mass of breeding age pigs is needed to maintain a feral pig population. Their tendency to visit dependable water sources contributes to their survival since this increases the mating chances of the population.

- d) Aggressiveness. Feral pigs are notoriously hostile and aggressive. This both aids and inhibits their ability to establish and survive in the wild.

3. Ecological interrelationships: 3

- a) Importance to community. Feral pigs are voracious omnivores that are able to be free-living in very rough country. They produce holes as they root that collect surface water and contribute to the shallow water ecosystem.
- b) Niche specificity. Feral pigs occupy a fairly ecological narrow niche.
- c) Extent of geographic range. Moderate geographic range, extending through the southern half of the U.S.
- d) Habit. Free-living.

4. Potential for genetic change: 2

- a) Interbreeding population size. There has been a dramatic increase in the number of U.S. premises where wild pigs are stocked and hunted. Although they are capable of breeding with domestic pigs, that is not highly likely. They interbreed quite well in the wild if animal numbers are adequate.

- b) Genetic stability/mutagenicity. There is a rather heterogeneous and broad base of genetic stock in the U.S. feral pig population.
- c) Potential for genetic exchange. Because they are not confined or managed, the potential is quite high that they will exchange genetic information with other feral swine in close enough proximity.
- d) Degree of genetic diversity. Feral pigs offer a genetically diverse population because of the history of their evolution.

5. Potential for monitoring and control: 3

- a) History of use and control. Some information is available on feral pig management.
- b) Accepted monitoring methods. Feral pigs are monitored by tracking, baiting, capture, and telemetric surveillance. However, it is very difficult to capture all animals in an area or region.
- c) Accepted methods of control. These are the main characteristics of feral pigs that justify the assignment of safety condition of 3.0 (moderate concern).
- d) Control of inadvertent release. Difficult to find and catch.

Evaluation of safety condition:

Since it is difficult to capture all animals in an area or region, the safety condition must be rated 3.

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Foot and Mouth Disease Virus (FMDV) - Phillip A. O'Berry

Accessible environment: The study site is the Plum Island Animal Disease Center, Plum Island, New York. It is a USDA high security research facility surrounded by navigable water and off-limits to the public. It is a coastal island, 3 miles off the northern tip of Long Island. The accessible environment is the Center, Plum Island, and host animals reachable by prevailing wind currents. If the virus reaches the mainland, the accessible environment then becomes North, Central and South America. Normal cattle will be exposed to a genetically modified FMVD strain to determine its immunogenic spectrum.

1. Pest/pathogen status: 5

- a) Assess effects on accessible environment. Foot and mouth disease virus does not occur in the U.S., Canada, Mexico, or most of Central America. The U.S. has experienced 9 outbreaks of FMDV between 1870, and the last outbreak was in 1929. The 1914-1916 outbreak involved 22 states and the District of Columbia. Its last appearance in North America was in Canada in 1952. The effects on animals in the accessible environment and on the entire agricultural system would be devastating (Committee on Foreign Animal Diseases 1984).
- b) Potential for exchange of genetic information. This enterovirus is a member of the picornavirus group. First described in 1514, it is the first filterable agent known to cause disease in animals. Seven virus types have been described from 1922-1956 and more than 60 subtypes have been identified. This virus has exhibited a high potential for exchange of genetic information.

- c) Ecological characteristics which affect pest status. FMDV has a very broad host range, including cattle, swine, sheep, goats, wild pigs, wild ruminants, hedgehogs, armadillos, rats, nutria, grizzly bears, elephants, buffalo, and capybara. Other animals are also susceptible. Rare infections occur in man. Clinical signs of FMDV are similar to those of other vesicular diseases, making field diagnosis very difficult (Oxer 1972). Some evidence from the United Kingdom has been presented to support the theory that FMDV can move from island to island on wind currents (Sellers et al. 1977). The incubation period of FMDV may exceed 7 days and inapparent infections have been reported (Shahan and Traum 1956).
- d) Other. FMDV importation, possession, or use is prohibited or restricted by law and by USDA regulations (Biosafety in Microbiol. & Biomed. Labs, 1984).

2 Ability to establish: 5

- a) Known mechanisms of survival. The broad host range and existence of inapparent carriers greatly aids the survival of FMDV.
- b) Known mechanisms of dissemination. Although transmission occurs mainly by direct contact between infected and susceptible animals, indirect contact through infected animal products, inhaled air, and premises contamination can also transmit FMDV. In one instance in California, FMDV persisted on a premises for 345 days (Shahan and Traum 1956).

Semen from FMDV infected bulls may contain the virus before the appearance of clinical sign of illness.

- c) Effects of population size. FMDV has a very low infectious dose, although it has not been precisely determined.
- d) Aggressiveness. Aggressive characteristics have not been described for FMDV.
- e) Other. Although eradication methods are costly and quite disruptive, the virus has been eradicated from some countries in which it occurred.

3. Ecological interrelationships: 5

- a) Importance to community. No positive ecological importance to its community has been described for FMDV. All host animals are exterminated and destroyed when an eradication program is successful.
- b) Niche specificity. No niche specificity has been described for FMDV.
- c) Extent of geographic range. FMDV geographic range is worldwide.
- d) Habitat. FMDV lives in the cells of a wide range of host animals and can survive long periods of time outside the animal body.
- e) Other. -

4. Potential for genetic change: 5

- a) Interbreeding population size. FMDV can exchange genetic information with other FMDV types and has the potential to do so with other enteroviruses present in animals. The theoretical limit of interbreeding population size is 1.
- b) Genetic stability/mutagenicity. Significant mutations in FMDV have been described, mostly involving its protein coat which is composed of 60 copies each of 4 polypeptides. Seven immunologic types have been described.
- c) Potential for genetic exchange. FMDV has a high potential to exchange information with other enteroviruses.
- d) Degree of genetic diversity. Extensive genetic diversity has been described among the more than 60 subtypes of this virus.
- e) Other. -

5. Potential for monitoring and control:

- a) History of use and control. Methods and procedures exist to monitor, control, and eradicate FMDV when it is detected. These methods are extremely costly and disruptive and are not always successful.
- b) Accepted monitoring methods. Extensive surveillance of imported animals and animal products and international travelers must be maintained. All cases of animals with blisters in the epithelium of

the mouth and feet must be reported to USDA officials immediately. Methods are well established to diagnose FMDV.

- c) Accepted methods of control. FMDV eradication methods consist of slaughter of infected and exposed animals and disposal of their carcasses by burial or incineration. Infected premises are decontaminated. Vaccination may or may not be used (Graves 1979).
- d) Other. A worldwide monitoring and reporting system is in place for this disease.

Evaluation of Safety Conditions:

There are compelling biological, ecological, economical, environmental, and statutory characteristics of FMDV and the disease that it produces to preclude its intentional release in the environment. Assignment of a safety condition of 5.0 (high concern) is justified.

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Loblolly pine (Pinus taeda L.) - Ron Sederoff

Accessible environment: Loblolly pine will be tested in Johnston Co., North Carolina, in an area adjacent to naturally occurring stands of pine. Pines will be allowed to flower and set seed during the experiment. Loblolly pine ranges from the coastal portion of the Atlantic Plain, Piedmont and upland provinces of the eastern and southeastern United States from Delaware, south to central Florida, west to eastern Texas and Arkansas and southern Tennessee. This region has long warm summers, relatively mild winters, and ample rainfall ranging from 40 to 60 inches per year. Pollen and seed dispersal could eventually allow spread of genes over a wide area.

1. Pest/pathogen status:

- a) Assess effects on accessible environment. Loblolly pine is not a pest or pathogen.
- b) Potential for exchange of genetic information.
- c) Ecological characteristics which affect pest status. None of the species with which loblolly pine interbreeds is a pest or pathogen. It is not likely to become a pest or pathogen unless a major genetic change caused a shift in its ecological competitiveness.

2. Ability to establish:

- a) Known mechanisms of survival. Loblolly pine could readily establish itself within its natural range and in many other locations. Its natural range is limited by drought and low soil pH in the west and by

cold damage to reproductive structures in the north, usually by late spring frost.

- b) Known mechanisms of dissemination. Loblolly pine males mature and release pollen grains for wind dissemination. Pollen dispersal can be detected at distances of 2 miles, but the potential distances of dispersal are much larger. Pollination takes place after female cones develop and scales separate. Seed development occurs in the year following pollination, and has a high degree of dormancy.
- c) Effects of population size. Known for rapid growth on variety of soils, but grows best on wet, moderately acid soils. They produce large wood volumes on lands unsuited to intensive agriculture.
- d) Aggressiveness. -
- e) Other. -

3. Ecological interrelationships:

- a) Loblolly pine is the most important softwood species in the southern pine region, with over half the total pine wood volume. It is dominant on about 29 million acres. It is known for its rapid growth on a variety of soils, but grows best on wet, moderately acid soils. Its wood is valued for pulp and structural materials. Pines grow rapidly and produce large wood volumes on lands unsuited to intensive agriculture. Southern pines with hardwoods becomes more common inland.

Loblolly pine is a dominant pioneer arboreal species and a major

determinant of the ecology in southern forests and in commercial woodlots where it has been planted. It is susceptible to several pathogens and pests (Baker 1972, Hepting 1971). It serves as a host to a multitude of insect pests, most are insignificant in terms of damage or mortality, but the most serious are: southern pine beetle (Dendroctonus frontalis), pine engraver beetle (Ips spp.), pine tip moth (Rhyacionia spp.), seedling debarking weevils (Hylobius spp. and Pachylobius spp.), and cone and seed insects (Dioryctria spp. and Leptoglossus spp.). Loblolly pine is also the preferred host of the southern pine beetle, the most destructive insect pest for the species.

The major fungal pathogen of loblolly pine is fusiform rust (Cronartium quercuum f. sp. fusiforme). Loblolly is also susceptible to black root rot (Fusarium spp. and Macrophomina spp. and others), pitch canker (Fusarium monilaforma var. subglucans), root rot (Heterobasidium annosum), and heart rot (Phellinus pini and Phaeolus schweinitzii).

- b) Niche specificity. -
- c) Extent of geographic range. Loblolly pine ranges from the coastal portion of the Atlantic Plain, Piedmont and upland provinces of the eastern and southeastern U.S., from Delaware south to central Florida, west to eastern Texas and Arkansas and southern Tennessee.
- d) Habit. Loblolly pine is associated with several related southern pine species in natural stands and is believed to hybridize in nature with at least four of these species, Pinus palustris (longleaf pine), P.

echinata (shortleaf pine), P. serotina (pond pine), and P. rigida (pitch pine).

Natural loblolly pine stands and plantations provide habitat for a variety of game and nongame wildlife species. These species include white tailed deer, grey and fox squirrels, bobwhite quail, wild turkey, and rabbits. Pine seeds provide a major food source for many rodents and birds. Pine lands are the chief habitat for some birds such as the pine warbler, brown-headed nuthatch, and Bachmans sparrow. Old growth loblolly pine stands are importance to the existance of the red-cockaded woodpecker. Large loblolly pine trees are roosting places and nesting sites for many birds including osprey and bald eagle.

4. Potential for genetic change:

- a) Interbreeding population size. The natural population of loblolly pine is large and capable of extensive interbreeding.
- b) Genetic stability/mutagenicity. Loblolly pine is a species with a high level of genetic variability. Studies of inheritance show no indications of unusual instability or mutability (Dorman 1976).
- c) Potential for genetic exchange.
- d) Degree of genetic diversity.
- e) Other. -

5. Potential for monitoring and control:

- a) History of use and control. Loblolly pine has a history of widespread use in the U.S. and in many other countries of the world.
- b) Accepted monitoring methods. The size and slow growth of the plants make monitoring and control readily possible for plants that are not yet sexually mature. Sexually mature plants could disseminate pollen over considerable distances, but seeds would be more easily controlled. Plants could be mechanically pruned to prevent pollen or cone formation.
- c) Accepted methods of control. The high degree of genetic variation in individual plants allows recognition of specific genotypes as a method of monitoring containment and dispersal.
- d) Control of inadvertent release. Plants grown outside of the natural environment could be effectively contained by soil, climate, or by the absence of pines with which loblolly could interbreed.

Evaluation of safety condition:

Loblolly pine is not a pest or pathogen, but it is a plant with great ecological significance in the southeastern U.S. It is important in natural forests and in establishing ecology of plantations. Confinement of immature plants is easy, but special methods are required to prevent the production and dissemination of pollen and seed from sexually mature plants. Loblolly pine should be assigned to category 2 because of its ecological importance and the potential difficulties of confining pollen and seed dispersal. The unmodified organism is unquestionably compatible with the environment, but its importance in the ecology and the economy requires more concern with the consequences of genetic modification.

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Cardiochiles nigriceps - Fred Gould

Unmodified organism: Cardiochiles nigriceps Vierick (Braconidae)

Accessible environment. The intended release site for C. nigriceps is Clayton, N.C. Given the mobility of this organism, the environment accessible to a population that escapes confinement is the entire southeastern U.S. wherever its host, Heliothis virescens, occurs. (The rationale for this definition of the accessible environment of C. nigriceps will be evident from the following discussion of its biological properties.)

1. Pest/pathogen status: Low concern

- a) Assess effects on accessible environment. C. nigriceps is a member of the insect family Braconidae of the order Hymenoptera. All species of this family are parasites of other insects (Barror & DeLong 1964). All of the species closely related to C. nigriceps are parasites of Lepidoptera (Borror & DeLong 1964, p. 554). It has been studied in detail because it is a parasite of Heliothis virescens, one of the most destructive pests of annual crops in the U.S. (Kogan et al. 1978). Quantitative studies of the effects of C. nigriceps on H. virescens populations date back to 1926 (see Chamberlin & Tenhet 1926, Bibby 1942, Snow et al. 1966, Neunzig 1963, Lewis & Brazzel 1966). A number of studies have offered evidence that the host range of C. nigriceps is limited to the single species, H. virescens. Laboratory and field attempts to establish nigriceps on Heliothis zea, a noctuid

relative of H. virescens, were complete failures. Although C. nigriceps may oviposit into H. zea larvae, all of the eggs die within 48 hours (Lewis & Brazzel 1966, 1968). More recent studies have endeavored to explain C. nigriceps host specificity from a physiological perspective (Davies & Vinson 1986).

- b) Potential for exchange of genetic information. There are at least 8 species in the genus Cardiochiles. These species have not been studied in detail at an ecological or physiological level. Most parasitic insects have well defined species barriers, but some "species" of insects can produce fertile hybrids (Proshold & LaChance 1974). Since C. nigriceps is the only species in the genus collected in annual crops (Danks et al. 1979), it is unlikely to have contact with other species with which it could exchange genetic material.
- c) Ecological characteristics which affect pest status. Given the narrow host range of C. nigriceps and the pest status of its host, there is little if any reason for concern that C. nigriceps could become a pest. Even if C. nigriceps changed genetically to expand its range, it would be unlikely to attack beneficial insects. Almost all Lepidoptera (moths and butterflies) have herbivorous (plant feeding) larvae. If the host range changed enough that C. nigriceps attacked a lepidopteran that fed on a weed, some damage could be done (some Heliothines related to H. virescens specialize on evening primrose and ragweed [C. Mitter, personal comm.]). Given the high degree of host

specialization of C. nigriceps involving both restricted physiology and restricted host-finding behavior (Lewis & Brazzel 1966), such changes are likely to involve complex and fine tuned evolutionary adjustments, not random genetic changes.

d) Other. -

2. Ability to establish.

- a) Known mechanisms of survival. C. nigriceps has a few generations per season in the Southeast. It overwinters as a prepupa in the underground cocoon of H. virescens (Danks et al. 1979).
- b) Known mechanisms of dissemination. C. nigriceps is a robust insect and a strong flier (F. Gould, personal observation). It can track cues from its hosts and fly directly to them. Although no studies of long distance (100-500 km) movement have been made, it is not unreasonable to assume such movement of C. nigriceps, given proper weather conditions (Rabb & Kennedy 1979).
- c) Effects of population size. There are no field data on this. As with most braconid parasites, an unmated female of C. nigriceps can produce fertile male offspring. Since adult females live up to 24 days, it is theoretically possible for a single female to mate with her sons and establish a population.

- d) Aggressiveness. C. nigriceps can parasitize over 90% of the H. virescens larvae in a field (Chamberlin & Tenhet 1926). When a single H. virescens larva, in the field, is attacked by C. nigriceps and another parasite, Microplitis croceipes (Cresson), it is C. nigriceps that is usually the sole survivor (Lewis & Brazzel 1968).
- e) Other. C. nigriceps can be reared in the lab (Vinson et al. 1973), so some characteristics of a modified C. nigriceps could be assessed before release in the field.

3. Ecological interrelationships:

- a) Importance to community. As indicated above, C. nigriceps has a very narrow host range. To the extent that its abundance dictates the abundance of H. virescens, it may alter food chains. Given that many predators and parasites that occur in natural ecosystems also feed on H. virescens in managed systems, the population density of H. virescens can influence the density of these predators and parasites (and their hosts) in natural systems. Such indirect effects have not been carefully studied, but are not generally considered to be key factors determining the ecology of any natural system.
- b) Niche specificity. As indicated above, narrow.

c) Extent of geographic range. Geographic range seems to mirror the geographic range of H. virescens, which includes North and South America. Therefore, establishment is much more sensitive to the presence of its host than to abiotic factors.

d) Habit. As above.

e) Other. When pesticides are sprayed, the parasite's abundance decreases (e.g., Lewis & Brazzel 1968).

4. Potential for genetic change:

a) Interbreeding population size. The size of natural C. nigriceps populations must be large in order to cause high levels of parasitism in an abundant, widespread pest species. Unless a characteristic of a genetically modified C. nigriceps was intensely selected for (e.g., pesticide resistance), it would be unlikely to significantly alter the genetic composition of the natural population.

An exception would be the inundative release of millions of these genetically altered organisms to establish biological control (e.g., Stinner, Annu. Rev. Entomol., 1980).

b) Genetic stability/mutagenicity. Details of molecular genetics of this species are unavailable at this time.

- c) Potential for genetic exchange. Given the mobility of C. nigriceps, a released strain could certainly exchange genetic information with other populations.
- d) Degree of genetic diversity. Unknown.
- e) Other. -

5. Potential for monitoring and control:

- a) History of use and control. Pesticides have been shown to decrease C. nigriceps populations (e.g., Lewis & Brazzel 1968).
- b) Accepted monitoring methods. There are no routine monitoring methods other than assessing the level of H. virescens parasitism. This is labor-intensive and requires lab rearing of larvae to pupation.
- c) Accepted methods of control. Because of their size, they can be enclosed in fine mesh field cages. Although these cages do not offer full confinement, the stacking of three successively larger cages, one over the other, could work if care was taken in the construction.
- d) Control of inadvertent release. Inadvertant releases would be impossible to eliminate unless the release was in an area where H. virescens host plants did not occur. In the Southeast, this is not a common occurrence, since H. virescens feeds on over 14 families of plants (e.g., Neunzig 1963).

Evaluation of safety conditions:

Evaluation of relative importance of specific factors: Most important factors: 1) beneficial parasitoid, 2) narrow host range, 3) high ability to establish. Given factors 1 and 2, there seems to be little risk related to C. nigriceps' capacity for establishing in the accessible environment. Therefore, the overall characteristics of this unmodified organism are of low concern for the purpose of genetic engineering experiments. I would therefore assign C. nigriceps a safety condition of 1.0.

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TABLE 1.
EXAMPLES OF DETERMINING LEVEL OF SAFETY CONCERN
FOR UNMODIFIED ORGANISMS (1)

Organism	Pest/ pathogen status	Ability to es- tablish	Ecologi- cal rela- tionships	Potential for genet- ic change	Potential for moni- toring & control	Overall level of concern
<u>Zea mays</u> (maize)	1 (2)	1	1	1	1	1
<u>Bos taurus</u> (cattle)	1	1	2	2	1	1
<u>Butyrivibrio fibrisolvens</u> (rumen bac- terium)	1	1	1	1	1	1
<u>Brassica napus</u> (rapeseed) Southwest U.S.	1	2	1	2	1	1
<u>Brassica napus</u> (rapeseed) Pacific NW	3	3	3	4	1	3
<u>Cardiochiles nigriceps</u> (parasitic wasp)	1	4	1	1	3	1
<u>Drosophila melanogaster</u> (wild type lab strains)	1	2	1	1	3	2

(1) Information developed by the Agricultural Biotechnology Research Advisory Committee (ABRAC) Working Group on the Classification of Unmodified Organisms.

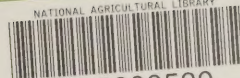
(2) Numbers in the body of the table represent the level of safety concern for the unmodified organism on a scale from 1 to 5. Level 1 represents the lowest level of safety concern and Level 5 represents the highest level of safety concern.

TABLE 1 (continued)

Organism	Pest/ pathogen status	Ability to es- tablish	Ecologi- cal rela- tionships	Potential for genet- ic change	Potential for moni- toring & control	Overall level of concern
<u>Pseudomonas</u> <u>fluorescens</u> 2-79 (3)	2	2	1	2	1	2
Soybean mosaic virus	3	1	1	2	1	2
<u>Pinus</u> <u>taeda</u> L. (loblolly pine)	1	3	2	2	2	2
<u>Sus</u> spp. (feral pig)	3	3	3	2	3	3
Imported fire ant	5	4	3	4	4	4
Africanized honey bee	4	5	3	4	4	4
Foot and mouth dis- ease virus	5	5	5	5		5

(3) The number "2-79" is part of the strain designation of the organism.

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